PARAMETRIC EVALUATION OF SORPTION OF COPPER

By

Ganoderma lucidum

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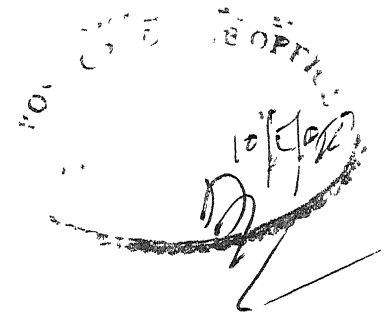

CERTIFICATE

Certified that the work presented in this thesis entitled "Parametric Evaluation of Sorption of Copper by *Ganoderma lucidum*" by Mr. Jose T. Matheickal has been carried out under my supervision and has not been submitted elsewhere for a degree.

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NOMENCLATURE

b	Langmuir isotherm constant
c_e	Concentration of sorbate in solution at equilibrium
c'	Predicted value of sorbate concentration in solution at desorption equilibrium
F	Fraction of metal bound as a proportion of the maximum metal binding capacity
K_f	Constant related to the sorption capacity in Freundlich isotherm
K'	Conditional stability constant
L	Ligand
L_b	Bound ligand concentration
L_f	Free ligand concentration
L_t	Complexation capacity
M	*G. lucidum*
M_b	Bound metal concentration
M_c	Alkali treated *G. lucidum*
M_f	Free metal concentration
$n:1$	Stoichiometry of metal:ligand complexation
q_e	Amount of sorbate sorbed/retained per unit weight of sorbents
q'	Predicted value of sorbate concentration is retained on the sorbent per unit weight of the sorbent at desorption equilibrium
Q_o	Langmuir isotherm constant
μ	Ionic strength.

ABSTRACT

Effect of pH and of presence of various anionic ligands (EDTA, oxalate, citrate, pyrophosphate) on Cu(II) sorptive capacity of Ganoderma lucidum (M) and its alkali derivative (M_C) was studied along with the evaluation of their complexation capacities to aid in elucidating the mechanism of metal uptake. The optimum pH of sorption was observed to be 6.0. The presence of phthalate ions in the buffer system reduced the metal uptake while acetate ions did not have any effect. Reversibility of sorption process was investigated employing the same sorption aqueous medium but without metal for desorption. A simple model, based on the adsorption isotherm and mass balance equation, was developed to predict the reversible component of sorption. The sorption process was found to be non-reversible while, eluants of 0.1 M HCl and 0.01 M EDTA could completely desorb the sequestered copper. Presence of anions inhibited the Cu(II) uptake, ranging from total inhibition at equimolar EDTA concentrations to apparantly no effect even at 20-fold molar excesses of pyrophosphate. The degree of inhibition followed the series EDTA > oxalate > citrate > pyrophosphate for G. lucidum (M) while the order was EDTA > citrate > oxalate > pyrophosphate for alkali treated G. lucidum (M_C). Employing pyrophosphate, instead of EDTA, to keep high metal concentration required in metal processing industries would make the end pipe pollution control method, using biosorption, more amicable for removal and recovery of copper. The conditional stability constants of the ligand(s) on the sorbent surface, responsible for metal uptake, were determined to be 4.5 and 4.7 L/mole respectively, for M and M_C, with a metal:ligand stoichiometry of 1:1. This gave further evidence on the influence of anions during metal removal by M and M_C.

Key Words: Heavy metal, biosorption, Ganoderma lucidum, reversible component of sorption, anionic ligands, complexation capacity.

1. INTRODUCTION

Human activity has inevitably increased the levels of metal ions in many of our natural water systems. Mine drainage, industrial and domestic effluents, agricultural runoff, acid rains, etc., have all contributed to some extent to the metal loads in these waters. As a result of their toxicity, environmental mobility and complex chemical forms, increasing attention is being directed towards studying their removal and recovery from metal bearing waste streams. However, these metals do not exist in isolation. Waste streams contain a variety of substances capable of forming complexes with dissolved metal ions. These complexing agents can be either inorganic or organic in nature. These resulting complexes can markedly alter the chemical form (speciation) of the metal in solution.

Due to the increased solubility of the metals in presence of complexing agents which are normally present in metal bearing wastes, it is often not possible to attain the required removal by chemical precipitation methods. Further poor settling of colloidial particles and slow reaction rates at low metal concentrations, make this method unattractive. Although, the recovery methods such as ion exchange, electrolytic removal or evaporation are more attractive, it is yet to be seen whether these methods are effective in the presence of complexing agents. Hence more attention has been directed towards the development of an alternate treatment strategy which involves cost effective commercial methods for the removal and subsequent recovery of these precious metals from the environment.

It has been known for sometime that heavy metal ions are accumulated by microorganisms, and a number of investigators have shown that microorganisms are valuable as an aid in removing and recovering metals from waste streams (Rucholoft, 1949; Cheng et al., 1975; Darnall et al., 1986). Unfortunately, because of the problems inherent in maintaining active microbial populations under the highly variable conditions of wastewaters, the living systems for metal removal and recovery are unreliable. However, certain types of microbial biomass can passively bind and accumulate metals even when metabolically inactive/dead, and can serve as a basis for development of a potent biosorbent material for concentration and recovery of strategic heavy metals (Muzzarelli et al., 1980; Volesky, 1987; Muraleedharan et al., 1988).

In order to establish biosorption as an alternative to the conventional metal recovery methods, the underlying mechanism controlling the extent and pattern of removal is to be well understood. Studies on the uptake of metals by biomass, however, have been complicated by the nature of both the adsorbent and the metal species in aqueous solution (Benjamine and Leckie, 1981). The cell walls of microbes contain many potential sites for the uptake of ions, and it is unlikely that any one type of molecule or functional group is responsible for the adsorption of these metals. Further many of the metals have complex solution chemistries and the equilibria involved are dependant on pH, concentration, the inorganic and organic anions present and many other factors (Volesky, 1987). Thus, localizing the metal deposition site within the biosorbent biomass and understanding

the metal sequestering mechanism, as well as elucidating the relevant metal deposition site, are all crucial aspects of the quest for an efficient biosorption process featuring high metal selectivity and uptake.

The present investigation is focussed towards the study of metal accumulation potential of a selected biosorbent under various aqueous conditions (e.g., pH) and in presence of different complexing ligands which are usually associated with the metals in waste streams. Further, the adsorption-desorption behaviour of the biosorption system has been investigated to examine the reversible component sorption as a function of metal concentration at a constant pH. An attempt to determine the conditional stability constants of the groups present on the sorbent surface has also been made. Ganoderma lucidum, one of the wood rotting fungi, which has recently been subjected for metal uptake potential and biosorption mechanism studies (Muraleedharan et al., 1988; Muraleedharan and Venkobachar, 1990) was selected as the biosorbent in the present investigation, and copper(II) was used as the model metal.

2. LITERATURE REVIEW

2.1. Scope

Metal uptake capacity of certain microorganisms can be used as the basis of novel attractive technologies for the treatment of metal bearing waste streams. Understanding the mechanism underlying this uptake is of prime importance in the process design considerations. The current information on the biosorption capacity of different microorganisms and the phenomena/mechanistic considerations are presented in the following sections. An account of the effect of chemistry of aqueous environment on the biosorption potential is also reviewed.

2.2. Accumulation of Metals by Microbial Cells

It has long been known that certain species of microorganisms can accumulate surprisingly large quantities of important metals (Rucholoft, 1949; Cheng et al., 1975; Darnall et al., 1986). Important metals in this case include, metals involved in toxicity to humans (e.g., the transport of cadmium or mercury through food chains) and metals of commercial and economic value (e.g., the recovery of silver from industrial waste solutions). Many defence strategies have been developed by these microbes to maintain a low cellular toxicant concentration. While some microbes have inherited the ability to resist high concentration of metal through their evolution under extreme environmental conditions, others have acquired a transferred resistance to a polluted environment consequent to industrial revolution (Wood and Wang, 1983). Some of the potential applications of this metal uptake capacity of microorganisms are discussed here.

2.2.1. Microbes in Metallurgical Applications: Bioleaching

It has been recognised that the microorganisms which developed 'strategies' for combacting effects of toxic inorganics can be used in metal bioleaching and ore beneficiation (Olson and Kelley, 1986). The most successful and widespread application of metal resisting microbes has come in the area of biohydrometallurgy where a significant portion of metal production comes from dump leaching of low-grade ores. Microorganisms such as Thiobacillus feroxidans and Thiobacillus thioxidans are found to be active in this process of metal leaching (Norris et al., 1980). Further, commercial production of uranium has been achieved through bioleaching process. Uranium bioleaching processes rely on T. feroxidans to maintain elevated levels of Fe^{3+} in leaching solutions (Olson and Kelley, 1986). Ehrlich (1986) reported the ability of T. feroxidans for non-selective leaching of silver from a mixed sulfide ore containing Ag, Pb, Zn, Fe and Sb in a batch process.

2.2.2. Metal Removal in Biological Waste Treatment Processes

The success of metal bioleaching methods has led to interest in the removal and recovery of toxic and valuable heavy metals from aqueous waste streams. Decontamination of mining and smelting wastewater has been reported, as the water passed through meandering streams and ponds where the metals were enriched in algal blooms (Hasset, 1979).

Microorganisms like bacteria, algae, fungi and yeast function either by accumulation of dissolved or particulate metals or by production of byproducts, which render the metal

insoluble. Rucholoft as early as in 1949 observed that activated sludge efficiently removed plutonium-239 from contaminated water. It was described that the decontamination process as the propagation of a microbial population having gelatinous matrices with tremendous surface area that are capable of adsorbing radioactive materials. The removal of heavy metals from municipal and industrial wastes by biological treatment systems has continued to be of greater interest (Cheng et al., 1975; Neufeild and Hermann, 1975). Hatch and Menawat (1979) reported that Sphaerotilus natans, a bacterium found in waste sludge and polluted waters accumulated iron, magnesium, copper, cobalt and cadmium in an external mucilage layer.

Norberg (1983) has reported the copper and cadmium accumulation capacity of Zoogloea ramigera, a dominant flocculating microbe of activated sludge.

Metal uptake capacity of an active microbial community consisting of Pseudomonas maltophilia, Staphylococcus aureus and a corneyform organism has been reported by Charley and Bull (1979).

2.2.3. Metal Removal by Microbially Inactive/Dead Sorbents

Although, the microbial populations in waste treatment systems can effect heavy metal removal, there is always a real danger that these metals may poison the system, stopping biological activity and microbial growth. Even if the contaminant's level in the effluent do allow microbial growth, organisms are often unstable in such an environment and over a period of time adaptation may lead to a change in their metal

uptake behaviour. Further, the system's inherent dependance on factors like pH, temperature and nutrient level etc., makes this method unreliable.

Separation of the biomass propagation and metal uptake steps is one logical solution to this problem and has led to the development of non-living biosorbent materials. This approach allows the control of conditions of growth and processing to increase metal uptake and facilitate elution and to improve physical properties of biosorbents. In fact, it has been demonstrated that dead cells accumulate heavy metals to the same or greater extent than living cells (Galun et al., 1983).

The use of waste biomass from fermentation industries as raw biosorbent material was investigated by Tsezos and Volesky (1981) who examined the uptake of uranium and thorium by Rhizopus arrhizus. Beveridge & Murray (1976), working with pure cell wall preparation of Bacillus subtilis, reported that the microbial cell wall removed and retained ions of high-atomic number elements. Further, Shumate et al. (1978) reported rapid uptake of uranium from solution by resting Saccharomyces cerevisiae and Pseudomonas aeruginosa cells. Each microorganism was capable of accumulating 100 to 150 mg U/g dry weight.

Dead cells of Zooglorea ramigera have also been examined for use in metal accumulation process (Norberg and Persson, 1984). The biomass, consisting of an acidic polysaccharide, was used to accumulate copper, cadmium,uranyl ions and other cations.

Muzzarelli (1980) has examined the metal complexing abilities of fungal chitosan. The chitosan-glucan complex, extracted and deacetylated by treatment of Aspergillus niger biomass with sodium hydroxide, was found to sequester transition metals effectively. This method was considerably more effective than was purified chitosan of animal origin.

2.2.4. Metal Uptake by Other Microbially Based Sorbents

Recent investigations by Muraleedharan (1988) showed the excellent metal removal efficiency of a wood rotting fungus, Ganoderma lucidum, from metal bearing wastewater. The metal uptake was not significantly effected by the presence of other competing ions. The biosorbent could be reused for atleast 15 times without loss of efficiency.

Rao (1989) compared the Cu(II) uptake capacity of G. lucidum, waste Aspergillus niger from citric acid fermentation, and waste activated sludge from a laboratory unit. Using 4 gL^{-1} sorbent concentration and 0.5 mM Cu(II), uptake for G. lucidum was maximum (98%) followed by waste activated sludge (40%) and A. niger (14%).

2.3. Biosorption of Metals - Mechanistic Considerations

An important aspect of the practical utilisation of microorganisms for the removal and recovery of the metals is the understanding of the mechanism underlying the uptake.

The term biosorption is not specific with respect to the mechanism of uptake which may be via: 1) Particulate ingestion or entrapment by flagella or extra-cellular filaments, 2) active transport of ions, 3) ion exchange, 4) complexation, 5) adsorption and 6) inorganic precipitation (Andelman, 1973;

Shumate et al., 1978). While the first two mechanisms have been reported for living cells, the latter mechanisms have been reported for living and dead microorganisms (Shumate and Standberg, 1978; Tsezos and Volesky, 1981; Beveridge and Murray, 1980).

Biosorption can be described as the non-directed, physical chemical complexation reaction between dissolved metal species and charged cellular components, akin in many respects to ion exchange. Also the precipitation or crystallization of metals can take place at or near the cell and possibly subsequent to initial biosorption complexation (Tsezos and Volesky, 1982a; Pooley, 1982). Although, the detailed biochemical or physiological reactions that actually occur is not considered here, it is important to realize that the physiological conditions of the cells at the time of inactivation, the chemical state of the reactive sites on the cells and the chemical state of the metals to be sorbed onto non-viable cell.

In the subsequent sections cell wall characteristics of various types of microbes along with their interaction with metals, other cellular components capable of metal sequestering are dealt with. Investigations that were carried out to localise the metal deposition site within biosorbent biomass to understand the metal sequestering mechanisms are also mentioned. The study on metal-ligand-microbe interaction is very important as in many practical applications, bisorbents will have to face the metal-ligand complexes rather than free metal.

2.3.1. Metal Interactions with Cell Wall Components

There have been numerous studies in which metal accumulation was either demonstrated or implied to occur at the

cell surface or within the cell wall matrix. It has been generally assumed that surface accumulation is the result of complexation reactions between metal ions and the charged receptive constituents of the cell walls (Volesky, 1987). Without elaborating on the structural details, it appears that there are constituents in these cell walls which have the potential to complex metal ions. However, the wall composition is species dependant and also to some extent, is subject to the conditions under which the organisms are grown. For example, the protein and phosphate contents of yeast cell walls are significantly greater for organisms cultured at slow growth rate (McMurrough and Rose, 1967).

2.3.1.1. Metal Interactions with Cell Wall Components of Bacteria

It has been observed that a stoichiometric interaction, either ion exchange or complexation, is possible between the metal ions and active groups on bacteria such as phosphodiester(teichoic acid), phosphate, carboxyl (glycocides) and amine (amino- and peptido-glycosides and bound protein) on the polymers making up the cell wall (Beveridge and Murray, 1976). Studies by Doyle *et al.* (1980) and Beveridge and Murray (1980) have provided strong evidences that the carboxyl groups (of glutamic acid) in the peptido-glycans present in the cell walls of Gram-positive *Bacillus subtilis* are the primary sites of divalent metal complexation. This is in contrast to earlier studies (Heptinstall *et al.*, 1970; Hughes *et al.*, 1973), which suggested that the phosphate containing thechoic acid in the cell wall was responsible for metal

binding. More recently, Beveridge et al. (1982) demonstrated that teichoic and teichuronic acids are the prime sites for metal deposition in the cell walls of Gram-positive B. licheniformis.

2.3.1.2. Metal Interactions with Cell Wall Components of Yeast

The surface of yeast cells can also act as an ion exchange resin with rapid reversible binding of cations. Strandberg et al. (1981) have demonstrated the rapid uptake of uranium and postulated that the polyphosphate groups and the carboxyl groups on the cell surface of S. cerevisiae are active in metal complexation. The phosphoryl groups appears to form stable complexes with uranium while the carboxyl groups became involved only when the phosphoryl groups are saturated. Rothstein and Meir (1951) suggested that the surfaces of yeast cells contain reactive groups that are chemically similar to the high molecular weight polyphosphates which are responsible for uranium accumulation. However, it has also been established that the carboxyl groups of proteins can effectively complex uranium (Dounce and Flagg, 1949), and proteins are present in yeast cell walls.

2.3.1.3. Metal Interactions with Cell Wall Components of Filamentous Fungi

Biosorption of metals by the biomass of filamentous fungi has been mainly associated with the cell wall. The fungal cell wall is thought to have two main components: interwoven skeletal framework microfibrils, usually of chitin, embedded in an amorphous layer of proteins and various polysaccharides (e.g., mannans, glucans, and galactans).

The formation of a coordination complex between the metallic species and the chitin nitrogen or oxygen has been suggested (Tsezos and Volesky, 1981). They suggested that uranium biosorption includes at least three processes, including uranium coordination with the amine nitrogen of chitin, the adsorption of additional uranium in the cell wall chitin structure, and the precipitation of uranyl hydroxide within the cell wall matrix. In contrast, thorium accumulated primarily on the outer cell surface (Tsezos and Volesky, 1982a). The proposed mechanism included the co-ordination of thorium with the nitrogen of cell wall chitin and the adsorption of hydrolysed thorium ions at the cell surface, apparently by other cell wall constituents.

However, involvement of cell wall components other than chitin has been indicated by recent studies by Muraleedharan and Venkobachar (1990). Experiments conducted by them with a wood rotting fungus G. lucidum have shown that the amino groups in proteins or the chitin present in cell wall do not significantly contribute for the Cu(II) uptake. The electron paramagnetic resonance spectra studies on the uptake of copper(II) by G. lucidum have shown that the G. lucidum was having a very stable organic free radical (g = 2.003) and the cell matrix embedding this free radical appeared to be responsible for its metal removal. Further, Esser and Brunnert (1986) isolated a cadmium binding protein from the fruiting bodies of the mushroom Agaricus bisorpus, but the contribution of this protein to total metal uptake was only 20%, indicating the involvement of other group(s) as major contributor(s) to metal accumulation.

2.3.2. Metal Interaction with Other Components of the Cell

2.3.2.1. Intracellular Traps

The biosynthesis of intracellular traps for the removal of metal ions from solution is one of the strategies developed by microorganisms, so that the metal ion concentration does not reach toxic levels. One example is the biosynthesis of metalothionine and removal of Cd or Cu by this sulfhydral bearing protein (Wood and Wang, 1983). The synthesis of biopolymers appears to depend upon presence of either alkaline earth metals like Na, Ca, K, Si or heavy metals like Cu, Zn, Fe, Co, Mo and Cd. In the former case biopolymers having predominantly an oxygen donor matrix to bind alkaline earth metals and in the latter case the biopolymers having a nitrogen and sulfur donor matrix to collect the heavy metal ion is synthesised. This correlates closely with the predicted partitioning for elements in organic or inorganic matrices (Williams, 1983). Wood and Wang (1983) have developed througn selection, a nickel tolerant mutant of the Cyanabacterium synechococcus. This mutant could tolerate upto 2×10^{-4} M nickel sulfate which is attributed to synthesis of biopolymers. Mutants with intracellular trapping mechanism tend to bioconcentrate the toxic metals intracellularly to approximately 200 times over external concentration. This process, however, is mutually exclusive to that which binds or precipitates the metals extracellularly.

2.3.2.2. Efflux Mechanism

Some of the non-selective and non-essential heavy metals are accidently picked up by metal transport systems designed for the transport of ions such as phosphates.

The resistance of microbial cells to such non-beneficial metals like As, Sb and Cd has been shown to occur through evolution of cellular exclusion mechanism. In some organisms like Staphylococcus aureus and E. coli, resistance is induced by operon like system (an operon is a DNA region that codes for several enzymes in a reaction pathway). The resistance appeared to be mediated by an acquired plasmid (extra chromosomal DNA molecule). Two separate plasmids, namely, Cad-A gene codes for proteins that are involved in the efflux of Cd on the inside and two H^+ from outside the cell, while Cad-B gene is believed to be responsible for the synthesis of a Cd binding protein similar to metallothionine.

2.3.2.3. Precipitation at the Cell Surface

The precipitation of insoluble metal complexes occursthrough a biosynthesis of oxidising agents such as O_2 and H_2O_2 or membrane bound sulfate reductases. The reduction of metal sulfate to sulfide provides a means by which metals can be complexed and precipitated at the cell surface. It is reported that several bacteria have been found to precipitate Ag as Ag_2S at the cell surface (Pooley, 1982). Tsezos and Volesky (1982a) proposed that significant fraction of uranium accumulated within the wall structure of R. arrhizus by the precipitation of uranyl hydroxide.

Results of Wood and Wang (1983) have indicated that Cyanadium caldarium, a thermophilic green algae can prevent the entry of metals like Cu, Ni or Cr through the cell wall by an extracellular precipitation mechanism. The results indicated that the algae possessed a membrane - associated sulfate

reductase system, indicating that sulfide precipitation is a cellular detoxification mechanism.

2.3.3. Study of Metal-Microbe Interaction: Non-reversibility of Sorption Reaction

The extent of heavy metal sorption depends on many factors and can usually be rationalized within the framework of solution and surface chemistry. But less well understood is the desorption reaction which may give an insight into the mechanism of uptake.

The conventional desorption tests for heavy metals are extractions using concentrated solutions of strong chelating agents like EDTA or concentrated acids or alkalies (Kuyucak and Volesky, 1989; Otter et al., 1989; Muraleedharan, 1988). This is in contrast to the usual desorption tests for organic chemicals in which the same aqueous phase is used for both adsorption and desorption to directly test the reversibility. Heavy metal desorption into various extraction solutions has been found to be incomplete, suggesting that the reaction is not completely reversible (Pickering, 1980; Stephenson et al., 1987). The usual finding is that substantial quantities of the heavy metals remain associated with the solid phase even at high extractant concentration that should displace all the physically adsorbed metal (Forstner and Wiltman, 1979). Studies have not been conducted on desorption that include adsorption and desorption isotherms using the same aqueous phase. However, Ditoro et al. (1986)have analysed the adsorption-desorption isotherm data and examined the reversible component sorption as a function of pH, ionic strength and particle concentration, using the same

aqueous phase. By studying the adsorption-desorption behaviour of nickel and cobalt-montmorillonite system in the same aqueous phase, they demonstrated that reversibility was not complete and varied as a function of chemical (pH and ionic strength) and physical (particle concentration) variables.

2.3.4. Study of Metal-Ligand-Microbial Sorbent Interactions

Generally, metal rich industrial effluents contain soluble organic or inorganic ligands to keep the high metal concentration in solution. These ligands are capable of forming complexes with dissolved metal ions and the resulting complexes can markedly alter the chemical form of the metal in solution thereby altering the sorption behaviour (Benjamine and Leckie, 1981).

There have been numerous studies of metal adsorption of 'simple' systems, where the only reactions in which the metal ions participate are adsorption and hydrolysis and it was shown that pH was the dominant solution parameter controlling adsorption in those systems. However, when complexing ligands are added to a system the results cannot be generalised easily. Metal adsorption sometimes increases (Benjamine and Leckie, 1981) and sometimes decreases (Tobin et al., 1987) depending on the particular metal, ligand, adsorbent and the pH range being studied. The interactions between metal ions, complexing ligands and the adsorbents may be divided into three categories (Benjamine and Leckie, 1981), i.e.,

1. metal-ligand complex may form that are non-adsorbing or weakly adsorbing, resulting in a decrease in metal adsorption,

2. the ligands may interact with the adsorbent so as to enhance or decrease the metal crystal uptake potential and
3. the metal-ligand system may form the complexes that are more strongly adsorbing than the free metal so that ligand presence results in enhanced metal uptake.

Tobin et al. (1987) reported that the presence of anions in solution inhibited the uptake of La^{3+}, Cd^{2+}, Pb^{2+}, UO_2^{2+} and Ag^{+} by Rizopus arrhizus biomass. The anions investigated caused a wide degree of metal uptake inhibition, and none enhanced metal uptake. EDTA caused the highest degree of inhibition and completely inhibited uptake of Cd^{2+} and Pb^{2+} at equimolar concentrations. Uptake of the other anions was also markedly reduced by the presence of EDTA. The extent of uptake inhibition caused by the anions and their reported stability constants followed the same order of magnitude, which showed a direct competition for the metal between the biomass and the anions. However, for certain La^{3+} and UO_2^{2+} systems these competition effects were replaced by adsorption of the complexed metals and for Ag^{+} system the anion inhibition of uptake was consistent with anion-biomass interactions.

Studies by Friiss and Keith (1986) on the effects of complexing ligands on uranyl biosorption have shown that, large excess of carbonate ions can strongly inhibit the uptake. Complexing ligands generally inhibited uranyl uptake in studies conducted with R. oligosporus biomass, and the behaviour was interpreted as indicating simple competition for the uranyl ions between the biomass and the ligands (Sears et al., 1984).

Studies by Benjamine and Leckie (1982), on adsorption by inorganic oxide surfaces, have shown that the metal-ligand complex may respond to changes in solution pH in variety of ways depending on the stereochemical arrangement at the sorbing surface. Further, pH effects the speciation of the metal in solution which in turn influences the sorption behaviour considerably.

Thus a quantitative knowledge of the metal species present in solution and of those associated with the cells is important for proper interpretation of the metal uptake behaviour of biosorbent materials.

2.3.4.1. Need to Study Chemical Speciation in Aqueous Medium

The major progress in the understanding of trace metal interactions with aquatic microorganisms over the past decade has come from a better appreciation of the importance of the chemical speciation of the metals in the medium (Morel, 1983). The speciation of a metal is a critical factor to consider when assessing the capacity of any microorganism to remove the metal from the aqueous phase. Further, it has been observed that the toxicity of several transition metals to microorganisms decreases as a result of complexation, indicating that the free metal ion is the toxic form (Allen et al., 1980), which in turn depends on the speciation of the metal.

2.3.4.2. Methods to Calculate Metal Speciation in Aqueous Medium

Some of the most sensitive analytical techniques e.g., neutron-activation analysis and atomic-absorption spectrophotometry, are not applicable to speciation studies because they measure only the total metal concentration (Florence

and Batley, 1977). Even though some analytical techniques do exist for speciation studies (e.g., differential pulse anodic stripping voltametry), they can only provide information about a few dissolved species. Furthermore, the very low levels of dissolved trace metals usually prevent accurate and reliable determinations of subspecies which occur at still lower levels.

Another classical approach to speciation consists of calculations based on chemical thermodynamic data. Several investigators (Perrin and Saycee, 1967; Florence and Bately, 1976) have used computer programs to predict the chemical forms of trace metals in a defined media. Those methods were based on the published stability constant data, together with known concentrations of various ions in the media to compute the equilibrium concentrations of the different chemical species. Bourg (1979b) has used a chemical equilibrium computer program, in which surface sites (for adsorption/desorption reaction) were treated as conventional ligands to describe adsorption effects in model solutions.

Of primary importance in determining the speciation of a given trace metal in solution are the nature and concentration of the various ligands present. Stumm and Morgan (1981) has reported that, if the 'available' ligand concentration is greater than the total trace metal concentration, the degree of complexation will be independent of the metal concentration and will depend only on the concentration of the individual ligands and the magnitude of the appropriate stability constant.

2.3.5. Studies on Chemical Complexation Capacity of Biosorbents

Measurement of complexation parameters (complexation capacity and conditional stability constants) can give an insight into the mechanism of biosorption by identifying the existence of probable groups responsible for metal binding (Kunkel and Manahan, 1973; Shuman and Woodward, 1973).

As the term implies, complexation capacity is a measure of the ability of a ligand to complex or mask the trace metal present. In other words, it is the concentration of the unbound ligand in a water sample and its value will depend on the metal to which it is complexed and the analytical method used for the measurement. In reality the measured stability constant is the conditional stability constant. That is, the constant is measured under a set of conditions (pH, ionic strength, competing complexation etc.) which make the value different from the thermodynamic stability constants (Morel, 1983). For a multi-ligand system, the measured stability constants will not be that for an individual compound, but rather will be an average value based on the overall extent of complexation (Crosser and Allen, 1977a).

In order to overcome the difficulties inherent in identifying and quantifying all the organic ligands present in the biosorbent material which are responsible for metal binding, several investigators have treated this surface sites as conventional ligands (Bourg, 1979b) and the complexation parameters was measured by grouping together these various ligands, and representing their behaviour by means of certain average properties (Stephenson *et al.*, 1987).

Most methods for measuring conditional stability constants involve a titration of the ligands with metal ion. The free metal ion concentration is measured and plotted as a function of total amount of metal added to the sample (Stephenson et al., 1987). The inflection or change of shape in the titration curve occurs when the concentration of added metal ion is equivalent to that of the ligand.

Many investigators have reported the existence of several types of complexing sites on the sorbent material. Mantoura and Riley (1975) reported the existence of number of complexing sites on humic acid extracted from soil, while Buffle et al. (1977) reported the existence of 1:1 and 1:2 metal:ligand complexes of copper and lead with ligands in natural waters. However, in most studies there have been no indication that the ligands present were sufficiently different to be treated independently in interpretation.

Recently, Stephenson et al. (1987), while investigating the mechanisms of the metal removal in activated sludge, discussed a method to find the existence of groups responsible for metal binding. The complexation parameters of these groups were determined by measuring the free and complexed or bound metal concentration with no direct measurement of either the bound or free ligand concentration. An assumption of 1:1 stoichiometry for the metal:ligand complexes, indicated the existence of two distinct groups of binding sites with different complexation parameters. But, with the consideration of n:1 stoichiometry where $n \neq 1$, improved complexation parameters were obtained. The value of 'n' was found to be less than unity in the copper ligand system.

2.4. Process Engineering Considerations for Metal Biosorption

Although biosorption of a variety of metals can be considered in the same framework as other adsorption processes, there are a number of significant differences that affect process design and scale up.

For this type of process to became generally useful it must offer some advantages over conventional ion exchange systems. Thus far, results indicate that biosorption process (that do not involve living microorganisms) is not as specific as ion exchange for particular metals so that its best use is for systems in which a range of metals are to be recovered (Olson and Kelley, 1986). Furtner, the mechanism of biosorption differs from one microorganism to another (Volesky, 1987). Since the microbial activity is not involved in biosorption process, the biomass from various sources can be combined in some instances. As in the case with ion exchange, the uptake depends on pH, solubility, the nature and charge of ionic species, other anionic species, temperature and the capacity of biosorbents. Further, the solution chemistry of the metal is to be well understood for the scale-up considerations. However, unlike ion exchange, there can be significant differences in how these parameters affect biosorption by various types of biomass. Thus, considerations should be given to properties of both the microorganisms and the metal, in scaling up biosorption process.

The biggest problems in scale up originate from the incomplete understanding of the relevant phenomena, although efforts to unravel some of the mechanisms continue. For example, the knowledge of chemical or physiological reactions that occur

during metal uptake might enable specification and control of process parameters to increase the rate, quantity and specificity of metal accumulation. Also, by knowing the inherent properties or activities of an organism that are responsible for metal uptake, it is possible to enhance the microorganism's ability to accumulate metals through environmental (i.e. growth conditions) or genetic manipulation.

2.5. Summary

Accumulation of metals by microbial cells can be used as an efficient, alternate strategy for the removal of heavy metal from metal bearing waste streams. But this process is strongly influenced by the local physical and chemical environment. Important parameters include pH and concentration of organic or inorganic complexing agents which can markedly change the sorption behaviour. Usually, the waste streams contain a variety of complexing agents and hence it is necessary to study the metal uptake potential of these biosorbents in such complex systems. Further, the metal binding site(s) or ligand(s) on the surface of the sorbents should be identified to aid in elucidating the mechanism of uptake.

3. SCOPE OF THE STUDY

From the preceding discussion it is evident that the biosorption potential of microbial biomass depends on many aspects of its chemical composition and the environmental conditions during the biosorption process. Further, as the waste streams contain a variety of complexing agents which can alter the chemical form of the metal, the solution chemistry of metals needs to be well understood to assist in assessing the application potential of these biosorbents to remove metals from complex, metal bearing industrial wastewaters. With this in view, the present study was undertaken. A wood rotting fungus, G. lucidum, was subjected to Cu(II) uptake studies. The studies were undertaken along the following lines.

a. Determination of equilibrium time for Cu(II) uptake by G. lucidum and treated G. lucidum.
b. Determination of equilibrium time for Cu(II) release from the biosorbents into aqueous phase of similar conditions as that of sorption process.
c. The effect of pH on metal uptake potential of biosorbents.
d. The adsorption-desorption equilibria to check the reversibility of sorption process.
e. Desorption of metal from biosorbents by a range of desorbents.
f. The effect of anionic ligands such as pyrophosphate, oxalate, citrate and EDTA on metal uptake by these sorbents.
g. Computer calculation of equilibrium concentrations of

species, present in mixtures of metal ions and complexing agents.

n. Determination of complexation parameters in the biosorption of Cu(II) by G. lucidum.

4. EXPERIMENTAL METHODOLOGY

4.1. Materials

4.1.1. Biosorbents

Ganoderma lucidum, a wood rotting fungus collected from Kerala, India, was used as the biosorbent in the present investigation. This non-edible mushroom grows on trees and is considered to be a plant pathogen. Identification of the specimen and microbial purity was ascertained by the Royal Botanical Garden (Kew, England). The hand picked mushrooms were washed in tap water, dried at 50°C and pulverised to a geometric mean (GM) size ranging from 300 to 600 µM.

4.1.2. Reagent Solutions

(a) Stock Cu(II) Solution: Copper solution of desired concentration range (0.20 to 4.0 mM) by dissolving AR grade $CuSO_4.5H_2O$ in distilled water.

(b) Stock KNO_3 Solution: Stock KNO_3 solution of 0.1 M was prepared using AR grade KNO_3 for use in the reaction mixture to have the desired ionic strength. 10% by volume of the stock solution was used in the kinetic and equilibria studies so as to give an ionic strength of 0.03 M. The ionic strength was determined by measuring the electrical conductivity of the solution sample and using it in the equation suggested by Benefield et al. (1982).

(c) Buffer Solutions: 0.2 M acetate buffers of pH 4, 5 and 6 were prepared as suggested by Lourie (1975). Phthalate buffers of same pH values and strength were also

prepared to study its effect in determination of optimum pH for biosorption.

(d) Stock Solutions of Complexing Ligands: Stock solutions of sodium citrate, sodium oxalate, disodium salt of ethylenediaminetetraaceticacid (EDTA) and sodium pyrophosphate, each of 0.1 M strength were prepared in distilled water.

4.1.3. Glassware

Glassware was saturated with Cu(II) before conducting the sorption studies by immersing in 1 mM Cu(II) solution for 24 hours. After that they were washed in 0.01 M HNO_3 followed by distilled water.

4.2. Methods

4.2.1. Pretreatment of Biosorbents

Alkali treatment of sorbents were carried out according to the procedure described by Muzzarelli et al. (1980). 100 ml of 40% NaOH was added to 20 g sorbent and the mixture was maintained at 103°C in a hot air oven for 4 hours. Sorbents were then washed repeatedly with distilled water followed by acetone and ether, and air dried. The dried sorbents were pulverised to the same size as the untreated one. Treated sorbent was designated as M_c.

4.2.2. Preconditioning of Biosorbents

Preconditioning of sorbents at the desired pH values was enforced in order to eliminate the effect of buffer components on copper uptake by biosorbent in some experiments. This was done according to the method suggested by Tsezos (1983). Sorbents were conditioned first by washing with distilled water. The washed sorbent was then suspended in water, the pH of

suspension was adjusted to the desired level with either 0.01 N NaOH or HCl solutions. Sorbents were allowed to stand for 3 to 4 hours following which the pH was again readjusted to the desired value and the process was repeated several times till there was no significant change in solution pH. Sorbents were then collected by filtration and dried at 65-70°C. After adding sorbents to the metal solution, the pH was again readjusted to the desired level, using 0.01 N NaOH or HCl, if necessary.

4.2.3. Estimations

4.2.3.1. Estimation of Total Soluble Copper(II)

Total Cu(II) estimation was carried out by "Cuprethol method" as given in Standard Methods (1968). The intensity of the yellow coloured complex was measured at 440 nM in a spectrophotometer-106 (Systronics, Ahmedabad). A calibration curve was prepared and with every estimation two standards of Cu(II) were used.

4.2.3.2. Estimation of Free Copper(II)

The free copper present in the solution was measured using an ion specific electrode (Orion, U.S.A.), connected to a digital pH/mV meter (Elico, Hyderabad). This electrode when in contact with a cupric solution develops an electrode potential across the sensing element which depends on the level of free cupric ion in solution. This potential can be measured against a constant reference potential with a digital pH/mV meter.

Different standards were prepared which bracketed the expected sample range and differed in concentration by a factor of ten. The calibration curve was prepared by plotting the

electrode potential of the standard solutions on a linear axis against their concentrations on the log axis. The concentrations of the samples were determined by comparing with the standards. Ionic strength adjuster (4% by vol., Orion Cat. No. 940011) was added to all solutions to ensure that samples and standards were of similar ionic strength.

4.2.3.3. Estimation of COD

COD of the solution samples were measured before and after the sorption experiment with various ligands, using close reflux titrimetric procedure as per Standard Methods (1985).

4.2.4. Experiments

4.2.4.1. Kinetics of Biosorption

Sorption kinetics studies were conducted at room temperature and at different pH values. A series of reaction bottles, containing 50 ml of 0.5 mM metal solution, 100 mg of sorbent, and 0.02 M acetate buffer to adjust the pH values in the range of 4-6, was mounted on a rotory shaker and agitated whereby each reaction bottle contents was in contact for a certain period of time. At the definite time interval a bottle was withdrawn from the shaker and the contents was centrifuged in a refrigerated centrifuge (Remi Instrument, Bombay) at 5000 rpm for 30 minutes (2000 x g) to remove the non-settled biosorbent. The supernatant was withdrawn and subjected to copper analysis.

4.2.4.2. Effect of Solution pH on Sorption

Batch sorption experiments were carried out at different pH values (4-6) to study the effect of solution

pH on biosorption. Experiments were conducted using both acetate buffer (0.02 M, pH 4, 5 and 6) and phthalate buffer (0.02 M, pH 4, 5 and 6) to maintain the pH. 50 ml of the reaction mixture, consisting of the sorbent (100 mg), metal solution (0.5 mM) and the appropriate buffer, was agitated on a rotory shaker at 30 rpm for 3 hour. Experiments at pH above 6 were not conducted because of the decreased solubility of copper. The contents of the bottles were centrifuged and the supernatant was analysed for Cu(II) residual concentration. Experiments were carried out using preconditioned sorbent also, to study the effect of anionic ligand components present in the buffer solutions in determination of optimum pH for copper removal. No buffer was used in this case and all other conditions maintained were identical.

4.2.4.3. Sorption-Desorption Equilibria Experiments

Adsorption-desorption equilibria studies were carried out by the following procedure. The aqueous phase (100 ml), containing buffered distilled water, metal of known concentration, ionic strength adjuster (0.1 N KNO_3) and sorbent (400 mg), was agitated on rotory shaker at 30 rpm until equilibrium was reached. The aqueous phase pH values were also varied in the range of 4 to 6 keeping the ionic strength constant (0.03 M).

The initial metal concentrations in the systems ranged from 0.2 to 4.0 mM for pH 4 and 5 and from 0.2 to 1.6 mM for pH 6. Sorbent-free blanks were run simultaneously to ensure that no precipitation of copper was occurred during the long

contact time. The contents were then centrifuged (5000 rpm for 30 min) and the metal concentration of the aqueous phase was determined.

For desorption studies, the solution mixture was filtered through a filter paper (Whatman No. 1) and the loaded sorbent was collected and dried at 50-60°C. The loaded sorbent (100 mg) was then resuspended in a copper-free aqueous phase (25 ml) of same conditions as that of sorption experiments. The reaction mixture was agitated until the desorption equilibrium was reached. Determination of desorption equilibrium time is discussed in next section. An aqueous phase sample was withdrawn after centrifugation and analysed for copper.

4.2.4.4. Kinetics of Desorption

The sorbents (2 g/L) were loaded with Cu(II) by contacting with aqueous phase containing 3.2 mM copper and agitating on a rotory shaker for 3 hour. The sorbents were then separated out, by filtration, and gently washed with distilled water. The desorption kinetics experiment was then carried out by resuspending the loaded sorbent (100 mg) in a series of reaction bottles containing 50 ml of buffered distilled water.

The pH was kept at 5 using acetate buffer during both adsorption and desorption experiments. The system was then agitated on a rotory shaker whereby each reaction bottle contents was in contact for a certain period of time. The supernatant was centrifuged and analysed for copper.

4.2.4.5. Desorption Studies Using Different Eluants

After Cu(II) has been sorbed onto the sorbent, from copper solutions of different initial concentrations, the sorbent was separated out by filtering through Whatman No. 1 filter paper and was gently washed with distilled water. The loaded sorbent was then dried at 50-60°C, and a known quantity (100 mg) was resuspended in the eluant solutions (50 ml). The mixture was agitated in a rotory shaker for 3 hours. The eluants used were 0.01 M disodium salt solution of ethylenediaminetetraacetic acid (EDTA) and 0.1 N HCl solution. The contents of the reaction mixture was then centrifuged and analysed for copper.

4.2.4.6. Effect of Complexing Ligands

Reaction mixture, consisting of 0.5 ml metal solution and respective complexing ion (citrate, oxalate, EDTA and pyrophosphate) was taken in different bottles and the pH was adjusted to 5 using acetate buffer (0.02 M). The concentration of each complexing agent was ranged from 0.01 to 1 X 10^{-5} M. The final volume of mixtures, in each bottle, was made upto 50 ml and the biosorbent (100 mg) was added. The entire mixture was agitated at 30 rpm for 3 hour, after which the contents was centrifuged and analysed for Cu(II) residual. The free metal concentration was measured using ion specific electrode (Orion, U.S.A.). Another set of bottles, with volume and contents of the mixture same as above except the complexing ligand, was also agitated at 30 rpm for 3 hour and the results were used as a control for the experiment. Cell of the aqueous

phase was also measured before and after the experiment to study whether the ligands or complexed species were adsorbed onto the sorbent surface.

4.2.4.7. Measurement of Complexation Parameters

Preconditioned sorbents (both treated and untreated) were used to determine the complexation capacity and conditional stability constants of the metal binding sites on the biosorbent surface. Reaction mixture of 50 ml volume, containing metal of known concentrations and preconditioned sorbents, was agitated on a rotory shaker for 3 hour. The initial metal concentrations were varied from 10 to 100 mg L^{-1}. Sorbent-free blanks were run simultaneously as a control for the experiment. The contents of reaction bottle was centrifuged and the change in the supernatant pH was noted. The total metal and the free metal concentrations in the supernatant were analysed.

5. RESULTS AND DISCUSSIONS

Biosorption potential of microbial biomass depends on many aspects like type of active sites and their ionisation on the surface of biomass, its microporous structure and the chemical composition and environmental conditions of the aqueous phase in which metal is dissolved. Since wastewater contains a variety of complexing agents, their effect on the metal uptake capacity of the microbial sorbents needs to be studied for scale-up considerations. The present work is, thus, directed to evaluate the metal uptake potential of the native Ganoderma lucidum and its alkali treated derivative, at different aqueous environmental conditions (pH, presence of anions, etc.). Sorption-desorption equilibria was studied to determine the reversible component of sorption. The effect of various anionic ligands/complexing agents on Cu(II) uptake by the sorbents were determined. Further, the complexation parameters and the stoichiometries of soluble metal-sorbent interactions were also studied. These aspects are expected to assist in elucidating the mechanism of biosorption by G. lucidum.

5.1. Sorption Kinetics

Figure 5.1 presents the residual Cu(II) concentration profile with time for pH 4, 5 and 6, for both G. lucidum (M) and alkali treated G. lucidum (M_C). The Cu(II) removal was rapid with more than 90% of the total sorption occurring in 30 minutes. The effect of pH on rate of sorption was marginal, though the total sorption capacity was much higher at pH 6 than at pH 4.

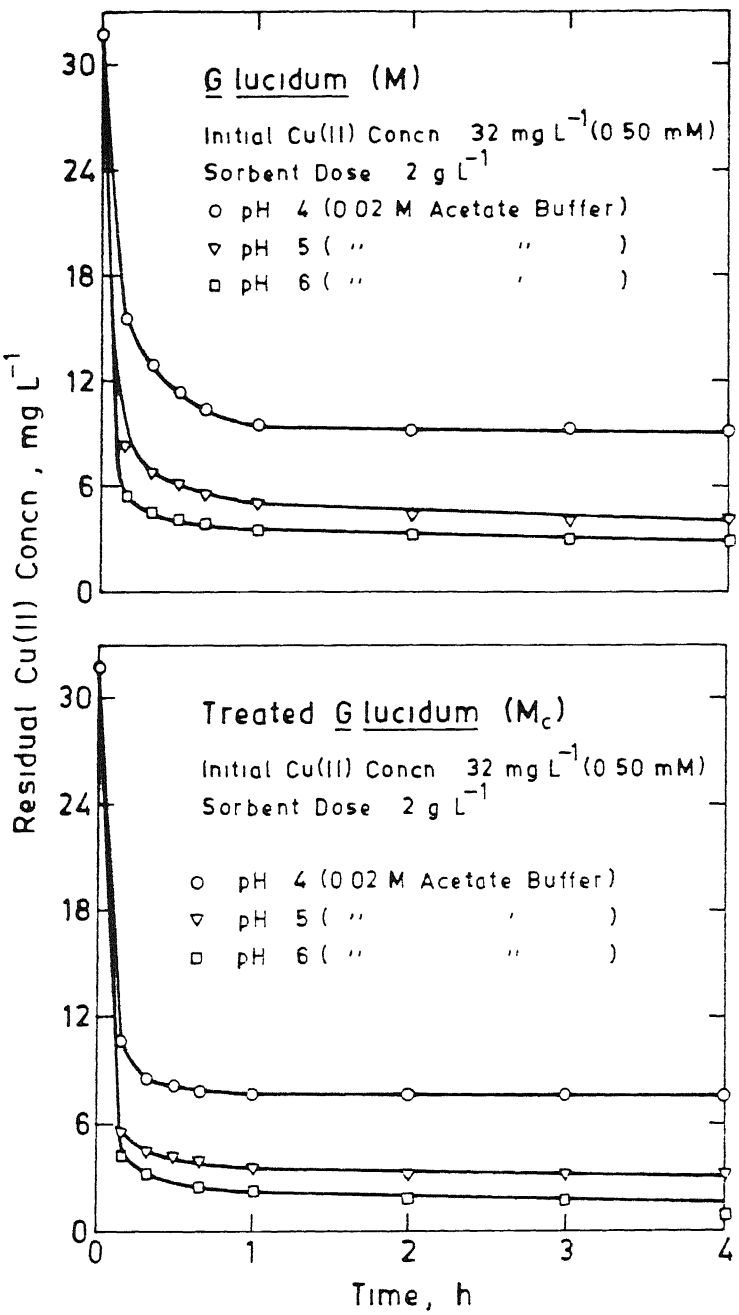

Fig 51 Kinetics of Cu(II) Sorption by M and M_c at Varying pH of Aqueous Phase

Adsorption has been described as a very fast phenomenon when it is not limited by mass transfer. Further, kinetics of adsorption depends upon its mechanism. The fungal cell wall can be viewed as a microporous structure because it allows the passage of intracellularly manufactured enzymes and other macromolecules. The kinetic data on Cu(II) biosorption in the present work clearly indicates that the equilibrium plateau is established within 60 min. The processes involved in reaching this must, therefore, be very rapid. Chemical complexation and adsorption are both rapid processes and may, therefore, account for the observed rapid establishment of equilibrium.

It is significant for the process application of Cu(II) biosorption that the examined Cu(II) biosorption system reached final equilibrium very quickly, independant of the solution pH. Hence, high rate contact processes such as fluidised bed reactor may be suitable, to make use of this rapid metal uptake by microbially based adsorbents.

5.2. Effect of Solution pH on Biosorption

Effect of hydrogen ion concentration on the binding of copper(II) to sorbents was studied in pH range of 4 to 6, as pH of most of the metal bearing wastewaters falls in this range. Furtner, in this pH range, as per solubility calculations, copper is expected to be in solution for an initial concentration of 0.5 mM. However, at pH 7 and higher, the effect of pH on metal uptake was not determined, as Cu(II) would precipitate as $Cu(OH)_2(s)$.

In the earlier investigations (Rao, 1989), to determine the optimum pH of biosorption of Cu(II) by G. lucidum, A. niger and the activated sludge in the range of 4 to 6, two different buffer systems viz., acetate and phthalate buffers, were used. The presence of acetate or phthalate as anions in sorption medium would be having their own effect on the removal of copper and, hence, will not exhibit truly the effect of only the hydrogen ion.

In the present investigation experiments were, therefore, conducted using acetate or phthalate buffers separately to maintain the desired pH range. Figure 5.2 shows the percentage removal of copper(II) by both M and M_c at different pH values. The following observations can be made:

i) The removal of copper(II) both by M and M_c in the presence of phthalate as anion (used to maintain pH 4 to 6) was less than that when acetate was employed for maintaining pH in the same range, indicating phthalate to be inhibitory to Cu(II) sorption.

ii) The optimum pH is found to be at 6 for both M and M_c irrespective of whether acetate or phthalate buffer system was used. This was contrary to the results obtained when acetate was used to maintain pH 4 and 5 and phthalate for pH 6.

For both G. lucidum (M) and alkali treated G. lucidum (M_c), as the pH was reduced from 6 to 4, the Cu(II) removal efficiency was decreased by about 20%. This indicates significant competition for the active sites between the copper(II) ions and the hydrogen ions. Further, metal-sorbent interaction depends on

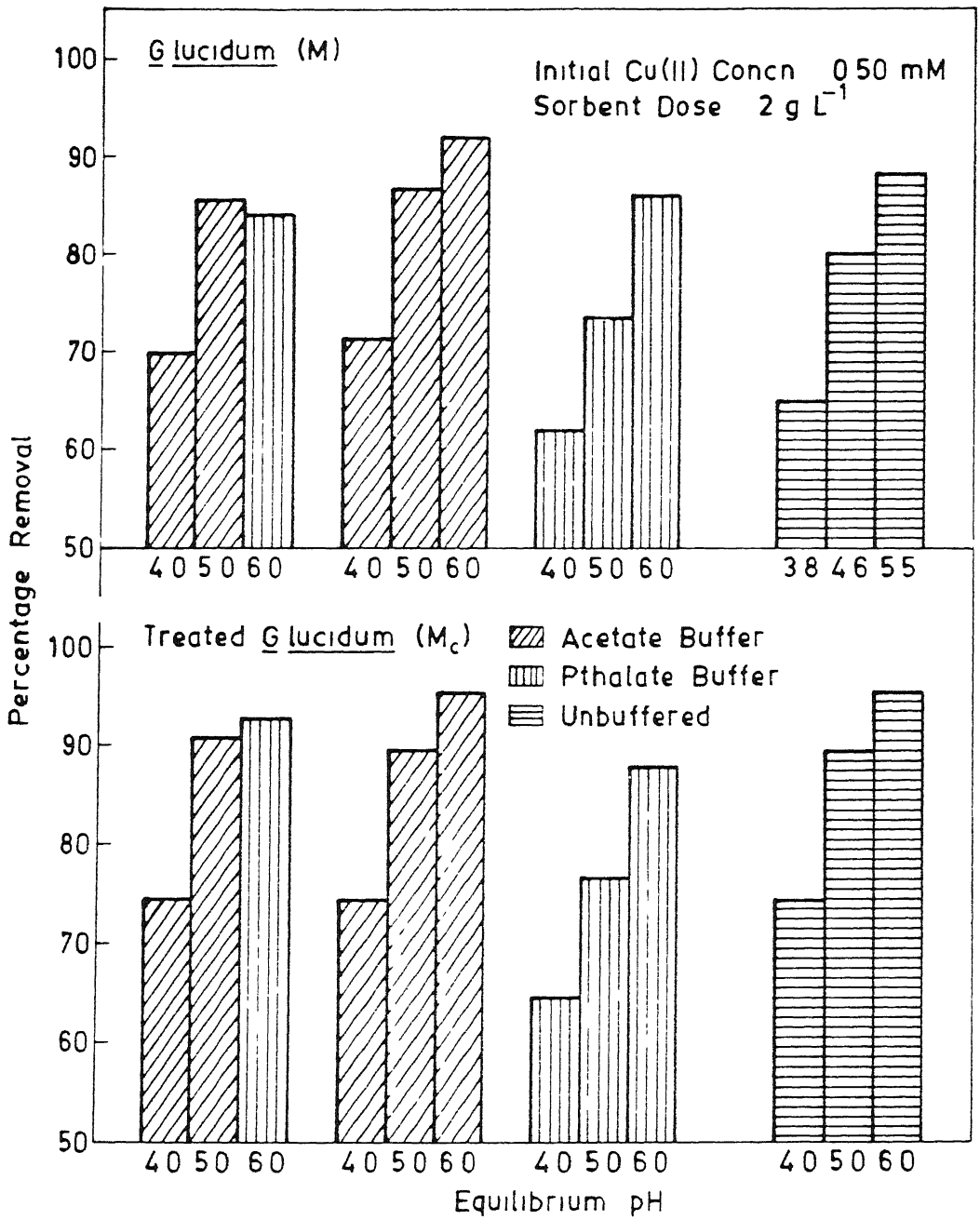

Fig 5 2 Effect of Varying pH and Buffer Systems on Cu(II) Removal by M and M_c

the speciation of the metal in the aqueous phase as well as the functional groups available on the sorbent. Since ionisable groups such as - COOH and $-NH_2$ may be involved in metal complexing P^{Ka} of these groups on the sorbents play an important role in the metal uptake. However, between pH 5 and 6, the pH effect was marginal and hence the biosorbents could be effectively used for industrial wastewaters where the pH usually ranges from 5 to 6.

In order to determine the absolute effect of presence of anions of the buffer systems, the sorbents preconditioned in the pH range of 4 to 6, were subjected to metal uptake studies without addition of any buffer. Results presented in Figure 5.2 show that the adsorption of Cu(II) was markedly affected by phthalate while acetate had no effect on the sorption behaviour by the biosorbents. The equilibrium pH of the solution, when G. lucidum (M) was employed for sorption process, appears to reduce slightly while it is not so with treated G. lucidum (M_c). In all further experimentations, whenever the pH was required to be kept constant, acetate buffer was employed.

5.3. Sorption-Desorption Equilibria: Studies on Reversibility of Biosorption

The partition of trace metals between solid phases and natural waters is an essential feature of their geochemical behaviour in the environments. Adsorption and desorption together determine and are equally important for the reversibility of the sorption process. The extent of reversibility is of major importance if one is concerned with the release of adsorbed metal into solution during changes in chemical environment (e.g.

concentration gradient). Further, the desorption reaction can be used as an important tool to study the mecnanism of biosorption. To determine the reversibility, the same aqueous phase with and without the metal can be used for studying the adsorption and desorption.

5.3.1. Adsorption Equilibria Studies

Adsorption isotherm can often provide much information about the nature of the physico-chemical interactions involved in the surface immobilisation of toxic metals. Plots of equilibrium metal loading by the sorbent (q_e, $mg.g^{-1}$) against residual concentrations of metal remaining in the solution after equilibrium (c_e, $mg.L^{-1}$) are given in Figures 5.3 to 5.5 for different pH values. The initial soluble metal concentrations ranged from 0.2 to 4.0 mM for pH 4 and 5 while it was kept between 0.2 and 1.6 mM for pH 6 due to the possible precipitation of Cu(II).

The sorption curves indicate that the solution pH affected copper(II) biosorptive uptake. For both M and M_c, lower Cu(II) uptake was observed at pH 4 than at pH 5 and 6. Further, at all pH values, alkali treated G. lucidum (M_c) was shown to be marginally better biosorbent compared to the untreated G. lucidum (M).

The Cu(II) biosorption curves could be linearised by widely accepted adsorption isotherm models, namely those of Langmuir and Freundlich. Linearised form of Freundlich equation is

$$\log q_e = \log K_f + \frac{1}{n} \log c_e \qquad (5.1)$$

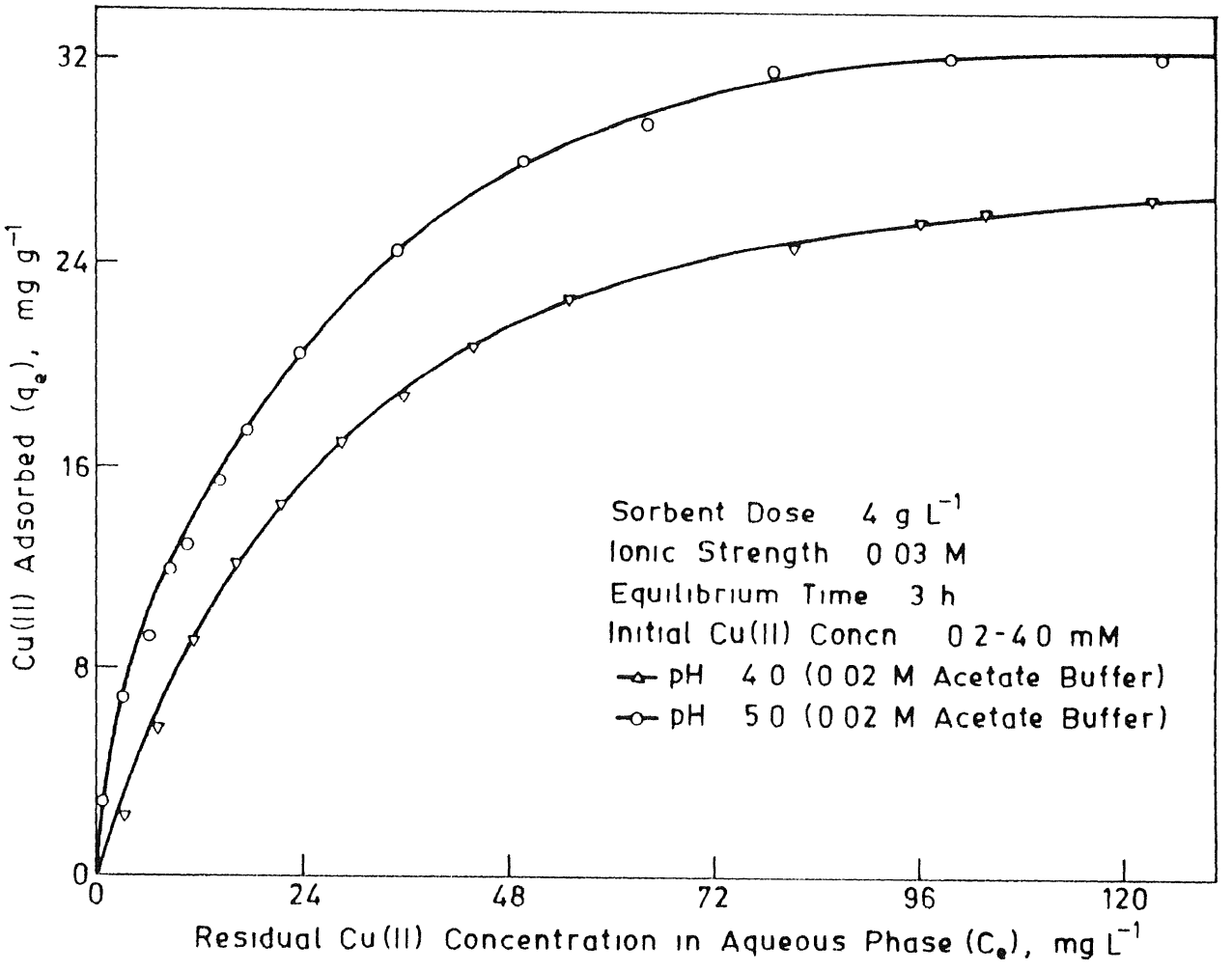

Fig 5 3 Equilibrium Distribution of Cu(II) Between Aqueous Phase and G lucidum (M) at Different pH

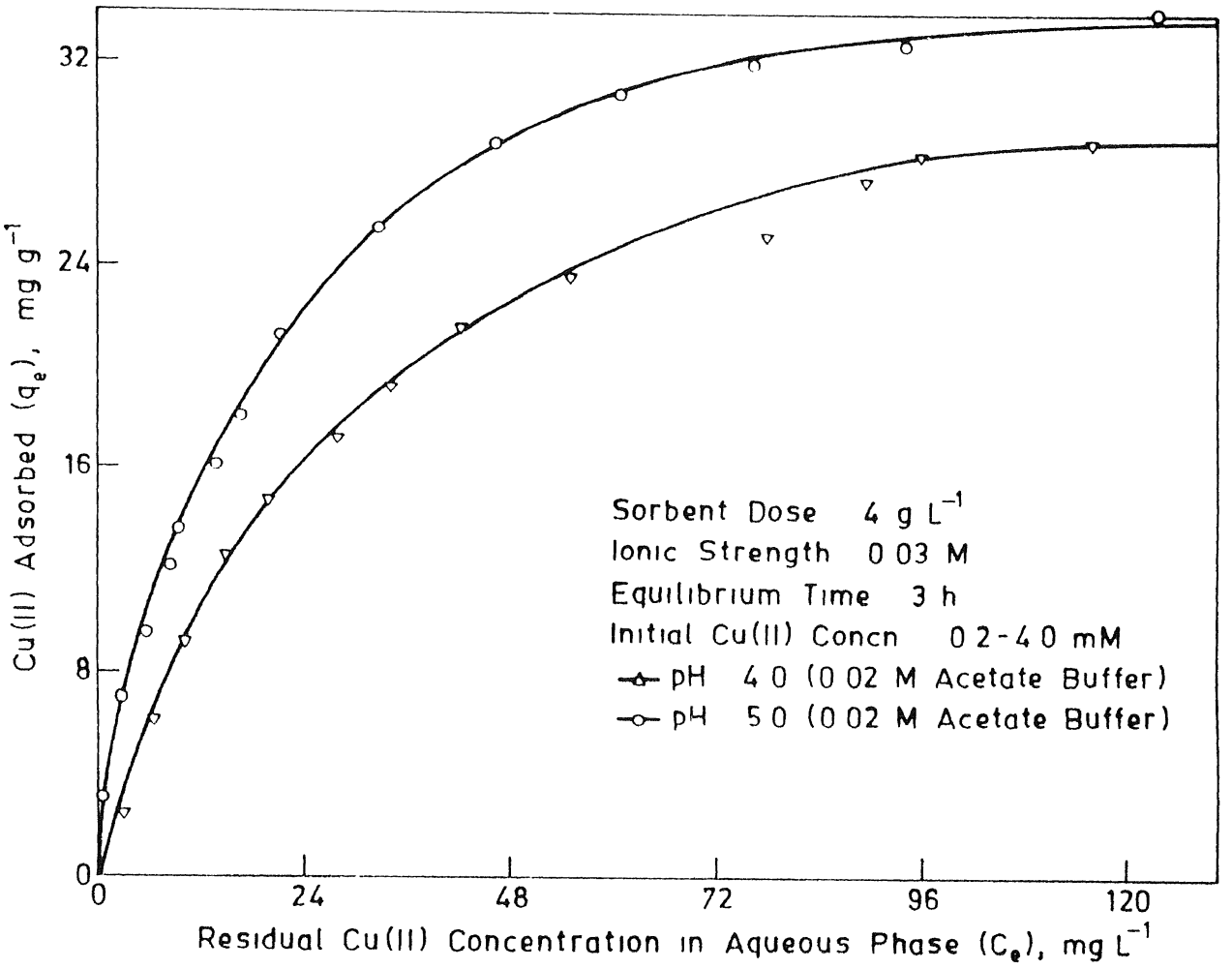

Fig 5 4 Equilibrium Distribution of Cu(II) Between Aqueous Phase and Treated G lucidum (M_c) at Different pH

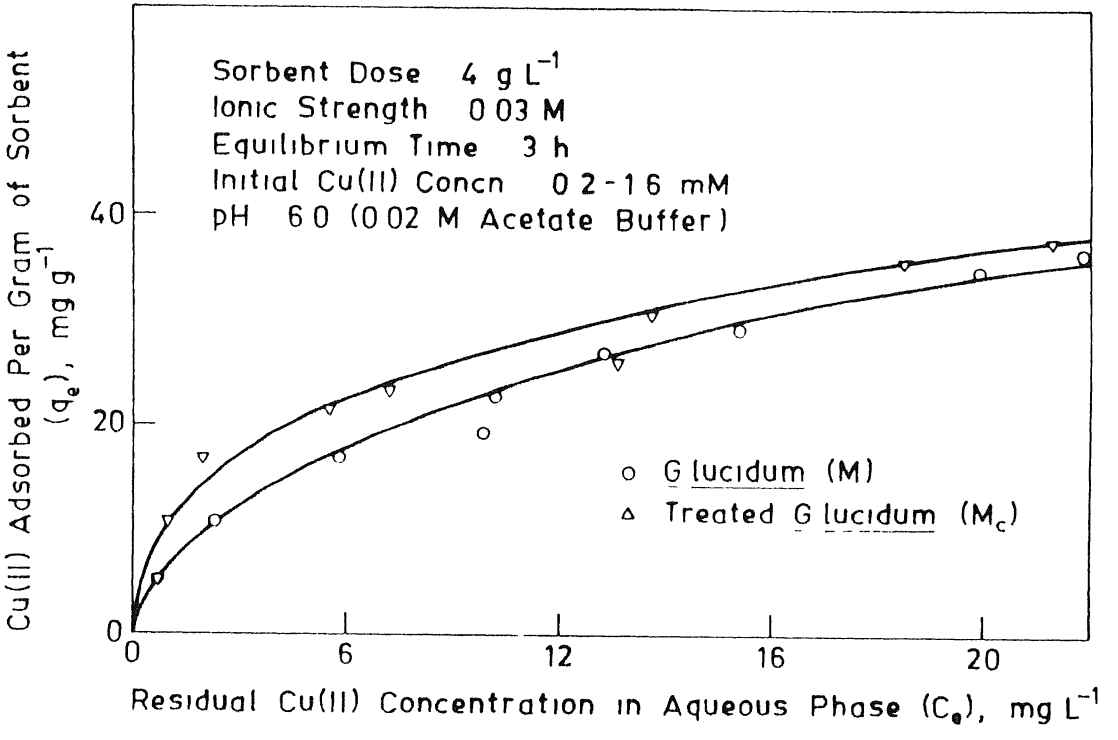

Fig 5 5 Equilibrium Distribution of Cu(II) Between Aqueous Phase and Sorbent (M & M_c) at pH 6 0

where q_e = amount of sorbate sorbed per unit weight of the sorbent; c_e = equilibrium concentration of sorbate in solution; and K_f and n are constants relating to sorption capacity and intensity.

Although, various linearised forms of Langmuir equation are available, the following relationship was used for the plot:

$$\frac{1}{q_e} = \frac{1}{Q_o} + \frac{1}{Q_o b} \times \frac{1}{c_e} \qquad (5.2)$$

where Q_o = weight of the solute deposited per unit weight of the sorbent; and b = a constant.

The experimental data on sorption equilibria for M and M_c (pH 4, 5 and 6; μ = 0.03) fitted to both Langmuir and Freundlich models are presented in Figure 5.6. However, slight variations of data from the Freundlich model was observed at higher initial metal concentrations. The isothermal parameters like K_f, $\frac{1}{n}$, Q_o and b are estimated and given in Table 5.1, along with the corresponding adsorption isotherm equations.

5.3.2. Prediction of Cu(II) Desorption Behaviour

The adsorption equilibrium isotherms along with mass balance can be used to make prediction regarding the reversibility of sorption reaction or desorption. This is because the desorption experiment simply involves replacing the equilibrium sorption medium with either distilled water or identical aqueous sorption solution without metal and waiting for new equilibrium. When the new equilibrium is established, obviously the entire metal will desorb, if the process is reversible.

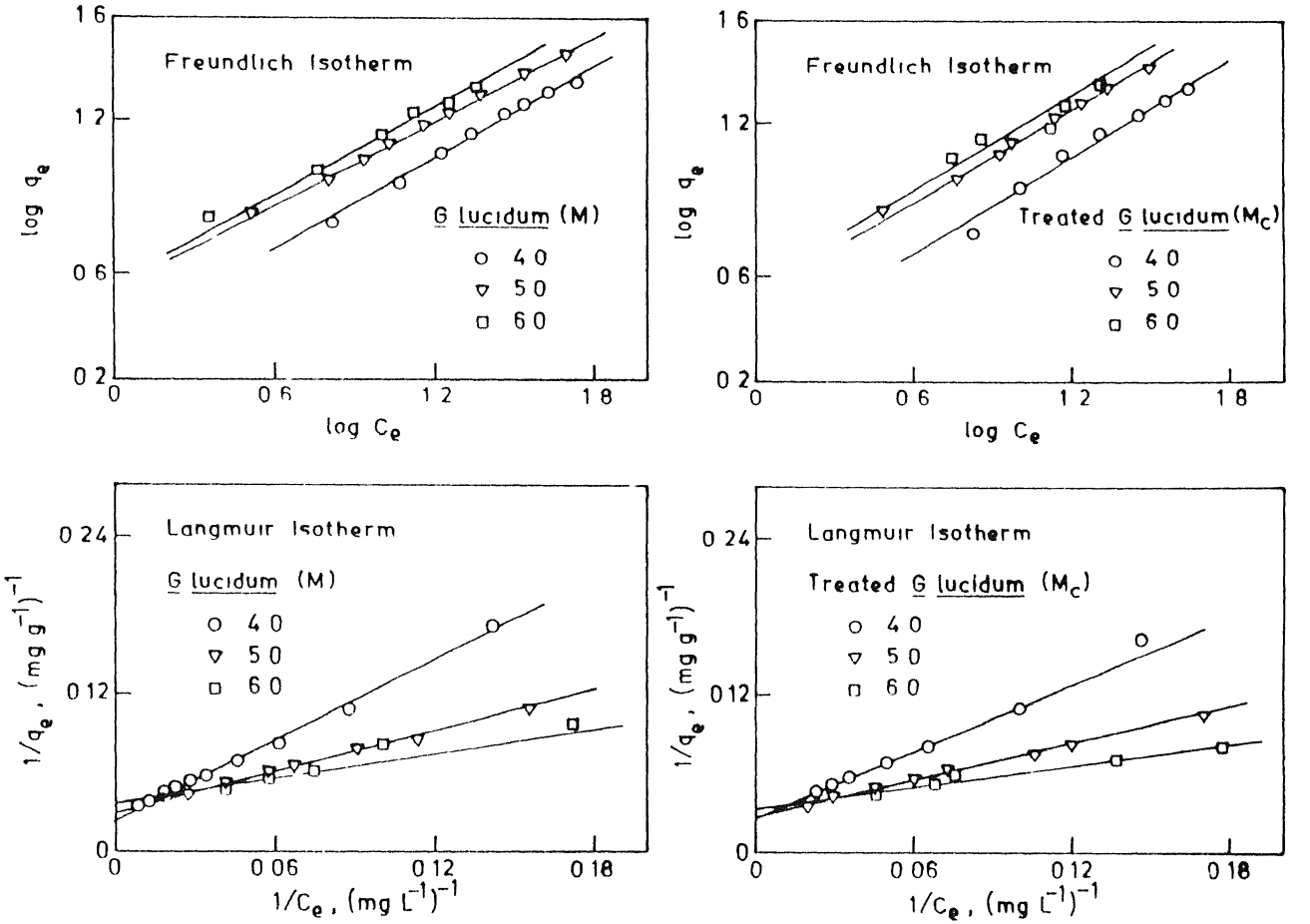

Fig 5 6 Linearised Cu(II) Biosorption Isotherms (Langmuir and Freundlich) for M and M_c

Table 5.1. Estimated Isotherm Parameters with Corresponding Biosorption Isotherm Equations for Sorbents (M) and (M_c)

Sorbent	pH	μ	Iso-therm	K_f	1/n	(Q^o)	(b)	Biosorption isotherm equation
G. lucidum (M)	4	0.03	L*	-	-	45.45	0.021	$q_e = 0.963\ C/1 + 0.021\ C$
		0.03	F**	2.188	0.60	-	-	$q_e = 2.188\ C^{0.60}$
	5	0.03	L	-	-	35.71	0.054	$q_e = 1.928\ C/1 + 0.054\ C$
		0.03	F	3.47	0.54	-	-	$q_e = 3.47\ C^{0.54}$
	6	0.03	L	-	-	25.1	0.129	$q_e = 3.24\ C/1 + 0.129\ C$
		0.03	F	3.47	0.652	-	-	$q_e = 3.47\ C^{0.652}$
Treated *G. lucidum* (M_c)	4	0.03	L	-	-	35.71	0.032	$q_e = 1.1507\ C/1 + 0.0322\ C$
		0.03	F	2.089	0.638	-	-	$q_e = 2.089\ C^{0.638}$
	5	0.03	L	-	-	41.66	0.048	$q_e = 2.028\ C/1 + 0.048\ C$
		0.03	F	3.31	0.630	-	-	$q_e = 3.31\ C^{0.63}$
	6	0.03	L	-	-	31.7	0.11	$q_e = 3.496\ C/1 + 0.11\ C$
		0.03	F	3.63	0.630	-	-	$q_e = 3.63\ C^{0.630}$

* L - Langmuir isotherm
** F - Freundlich isotherm.

Linearised Langmuir adsorption isotherms were employed to formulate the desorption model since the data gave a better fit for the entire range of initial metal concentrations.

Langmuir isotherm can be expressed as

$$q_e = \frac{b Q_o c_e}{1 + b c_e} \qquad (5.3)$$

Upon initial equilibration with the metal, the amount adsorbed, q_T, is:

$$q_T = q_e W \qquad (5.4)$$

where W is the biosorbent dose.

If the sorption equilibrium solution is replaced by either distilled water or by identical aqueous sorption solution without metal, the mass balance is

$$q'W + c'\forall = q_T$$

where q' and c' are the new equilibrium values and $\forall$ is the volume of solution. The value of c' can be determined from

$$q_T = c'\forall + \frac{b Q_o c'}{(1 + bc')} \cdot W \qquad (5.5)$$

The predicted c' value from the mass balance and the adsorption isotherm can be determined using the following equation,

$$b\forall c'^2 + (\forall + bQ_oW - bq_T)c' - q_T = 0 \qquad (5.6)$$

A comparison of experimental c' value with that predicted using the above equation, can facilitate to determine whetner the desorption is reversible or not.

To study the desorption of Cu(II) from the loaded sorbent into aqueous phase, the equilibrium time for desorption need to

be determined. The desorption kinetic experiments were conducted by determining the desorbed Cu(II) concentration into aqueous phase from the loaded sorbent (M and M_c) at various time intervals. The desorption kinetics behaviour by both M and M_c at pH 5 is shown in Figure 5.7. The kinetics of metal desorption was found to be rapid with equilibrium being attained just after an hour. However, a three hour equilibrium time was taken for desorption.

Experiments were conducted, to check the applicability of the desorption model developed for prediction of desorbed metal concentration, and to study the mechanism which prompts desorption. The predicted desorption isotherms, using the above model, and the experimental desorption isotherms at different solution pH (for M and M_c) are presented in Figures 5.8 and 5.9. It can be observed that the Cu(II) desorbed into the aqueous phase at equilibrium time is much less than the predicted values. This indicates that the sorption is irreversible. This, further, suggests that involvement of chemical interactions between the metal or its species and sorbent, is responsible for adsorption. Observed meagre reversible component of sorption could be attributed to the physically adsorbed copper. Further the results suggest a slight decrease in the reversible component of sorption as the pH increases. However, significant non-reversibility existed throughout the pH range (4 to 6) for both M and M_c.

As the desorption did not occur in the simple aqueous medium, the results of the experiments on desorption, conducted using aqueous based eluants like EDTA and HCl, are presented in the succeeding section.

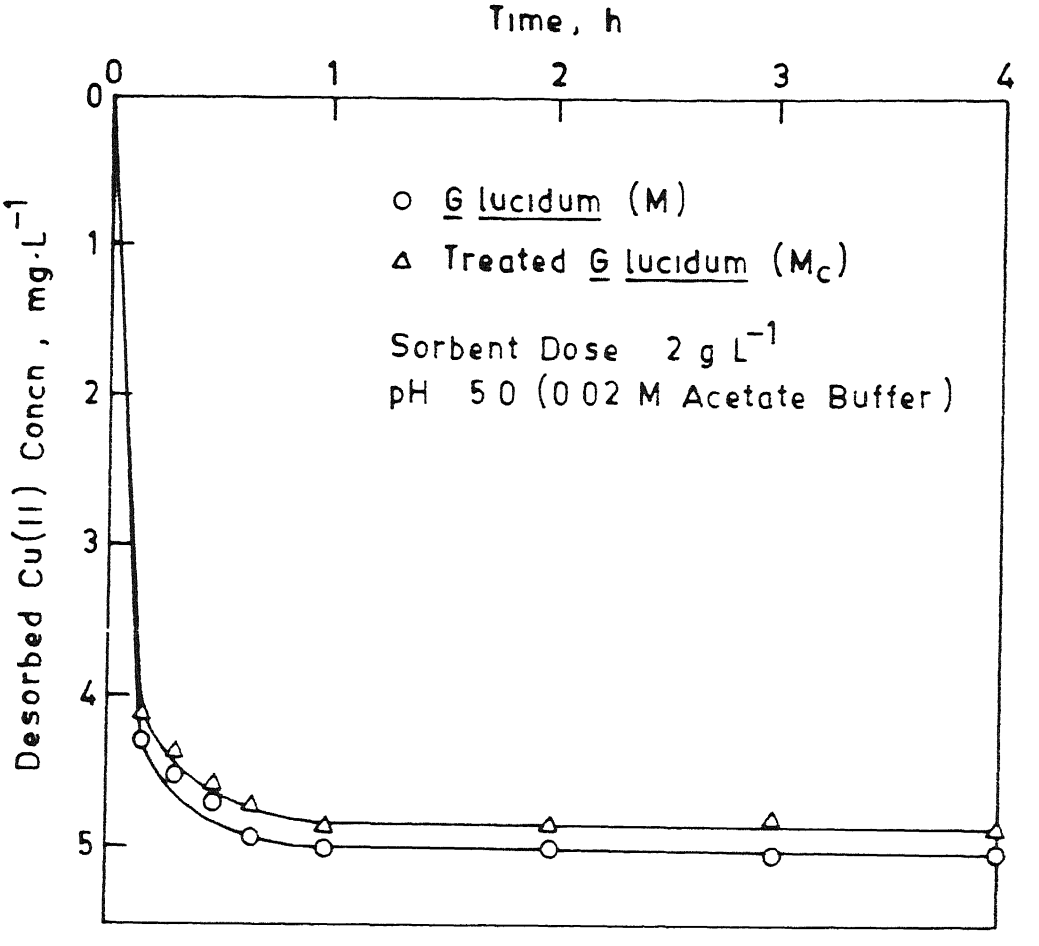

Fig 5.7 Kinetics of Cu(II) Desorption from M and M_c

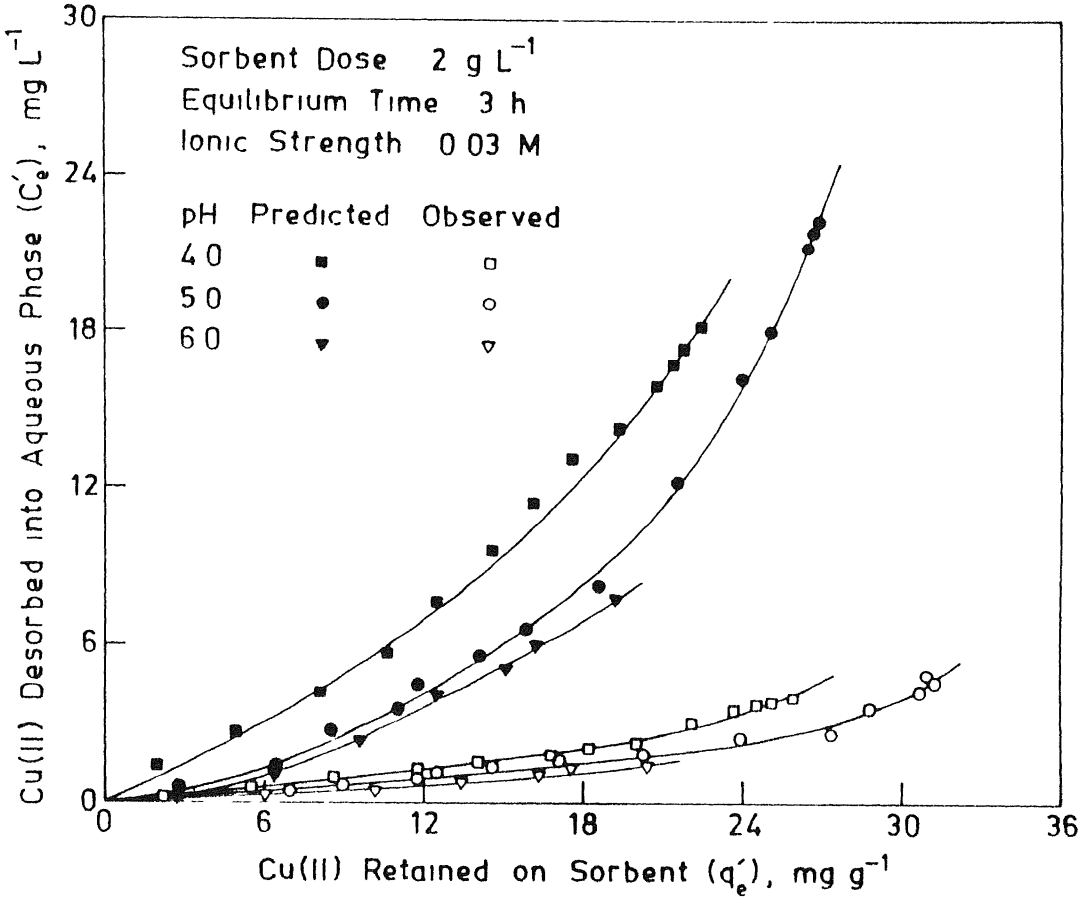

Fig 5 8 Equilibrium Desorption of Cu(II) into Aqueous Phase from G lucidum (M)

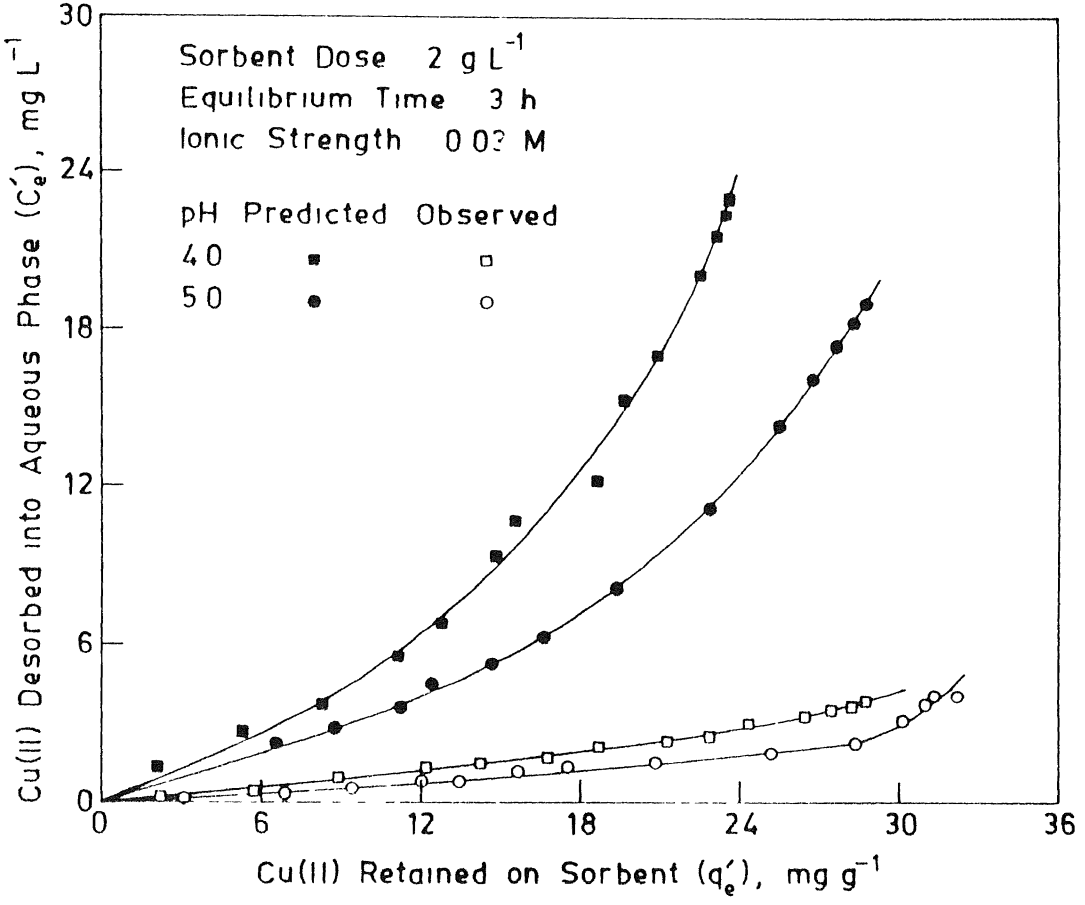

Fig 5 9 Equilibrium Desorption of Cu(II) into Aqueous Phase from Treated G lucidum (M_c)

5.4. Desorption of Cu(II) from Biosorbent Using Different Eluants

Two types of eluting agents, one a strong chelating agent of the metal (ethylene diamine tetra acetic acid) and another mineral acid (HCl) were tested and evaluated as to their desorption capacity for Cu(II) from the fungal biomass M and M_c. These biosorbents, earlier loaded with copper(II) at pH 5, were subjected to elution using 0.01 M EDTA or 0.1 M HCl for 3 hours at room temperature. A plot of Cu(II) adsorbed from aqueous phase against Cu(II) desorbed from solid phase for the two eluants is presented in Figure 5.10. It is evident that virtually all of the copper(II) adsorbed on the biomass, was desorbed during the elution process. Elution by 0.1 M HCl solution resulted in complete desorption of Cu(II) into the solution phase while slight amount of Cu(II) appears to have been retained on the sorbent after the desorption by 0.01 M EDTA. The elution efficiency of the same aqueous phase as that of sorption medium, but without copper, was also checked. The results given in Figure 5.10, suggest that the desorption of Cu(II) into the same aqueous phase is negligible.

In the case of HCl solution, the high concentrations of protons may dislodge Cu(II) from the active sites so as to make the bond between Cu(II) and prospective group(s) labile. EDTA forms a strong complex (stability constant of 10.7) with Cu(II) and the desorption mechanism may be of direct competition for the metal between the biomass ligands and EDTA.

In the course of present experiment, no attempt, however, was made to optimize the elution process. It is possible,

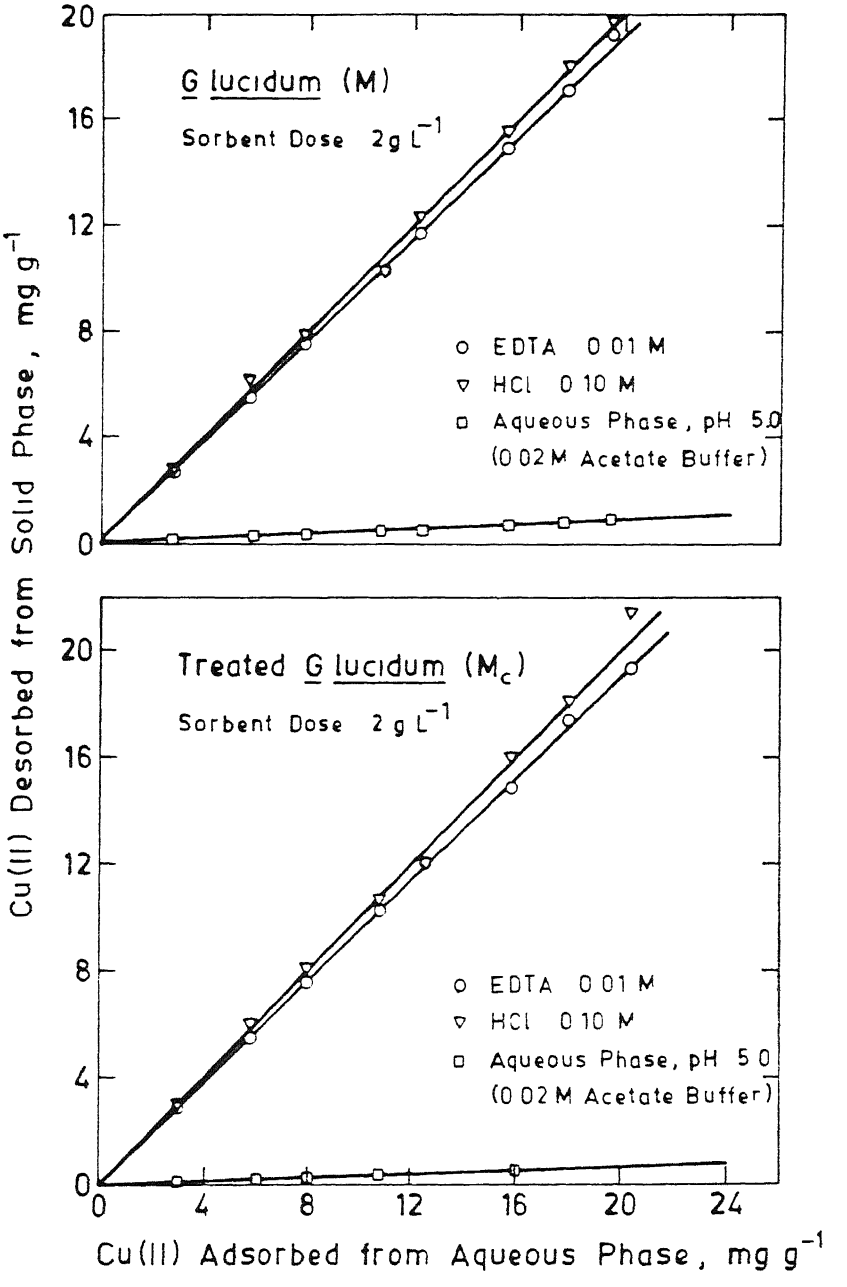

Fig 5 10 Desorption of Cu(II) from Solid Phase Using Different Eluants

however, in large scale operation, to optimize the elution process to produce a low volume, high concentration eluate to maximum copper recovery efficiency while minimizing the elution effects. This could be done by proper selection of EDTA or HCl concentrations and eluant volumes.

5.5. Studies on the Effect of Complexing Ligands on Cu(II) Sorption

Adsorption of trace metals from aqueous solution varies, depending on the physicochemical forms of the metal. This depends on the chemistry of water in which the metal is dissolved. Generally, metal rich industrial effluents contain soluble organic or inorganic ligands which aid to keep the high metal concentration in solution that is required for plating purposes. The complexation of metal ions with these ligands can dramatically increase or decrease adsorption compared to a ligand-free system. Further, the metal adsorption appears to be a highly species dependant phenomenon; for example, there may be abrupt change of adsorption behaviour coincident with the formation of a particular metal species. Thus, for the biosorbents to be effective for the treatmert of industrial wastewaters, their interaction with metals should be stronger than that of metal and soluble ligand complexes. Further, the percentage distribution of the metal species in the complex wastewater system should be known for the practical application of any treatment process. The present study evaluates the significance of the presence of ligands and their importance in process design considerations, by investigating the effect of few complexing

anions like pyrophosphate, oxalate, citrate and EDTA (some of these are often used in metal plating industries) on the sorption of Cu(II) by biosorbents. The speciation of the metal, in presence of various concentrations of these ligands and at a particular pH, was determined using a mathematical model, described in the following sections.

5.5.1. Speciation of Cu(II)

Calculation of Cu(II) speciation was accomplished using a modified version of the computer program COMICS (Perrin and Sayce, 1967). This quantitative approach to metal binding could give some useful informations, i.e.,

1. Estimation of equilibrium concentration of free metal ions, complexing agents and metal complexes, thus providing a useful picture of the distribution of each kind of species in a mixture of ligands.
2. The effects of variations in ligand concentrations on the speciation of metal complexes.

The program, COMICS (Concentration Of Metal Ions and Complex Species), was designed to deal with all types of metal-complex equilibria, such as mixed-ligand or mixed species, hydrolysed, protonated, or polynuclear species, and the usual stepwise (1:1, 1:2, etc.) complexes, as well as protonated ligands and hydrolysed metal ions. Though the speed with which this method leads to convergence was less compared to many other mathematical models, this was offset by the much simpler programming requirements and also by the greatly diminished amount of core storage. In fact, in its first application, COMICS was

used to compute the composition of a 10-metal-10-ligand system. In the present work, the program was simplified so as to use for one-metal-two-ligand systems. This program calculates the equilibrium concentrations of different species present at a particular pH, provided the total soluble concentrations of metal and ligand, the association constants of the species formed, and the metal-ion hydrolysis constants are known. These constants were adapted from Morel (1983).

5.5.2. Effect of Ligands on Cu(II) Sorption

The effect of some selected ligands of different aqueous phase concentrations, on the Cu(II) removal by sorbents (M, M_c) are presented in Figures 5.11 to 5.14. To obtain these, experiments were conducted keeping the solution pH constant at 5 by adding 0.02 M acetate buffer, and hence the system immediately became a one-metal-two-ligand system out of which acetate was common. The percent equilibrium concentrations of the different metal species present in the aqueous phase, as calculated by the computer program, are shown along with their percentage removals by M and M_c in Figures 5.11 to 5.14. The computer predicted free metal concentrations in the presence of ligands were in complete agreement with the measured free metal concentrations using Orion ion specific copper electrode.

An examination of the figures indicates that, in general, the anions investigated caused a wide degree of inhibition of metal uptake and none enhanced the metal uptake. Pyrophosphate, even at the highest concentrations of 10 mM (metal-ligand ratio

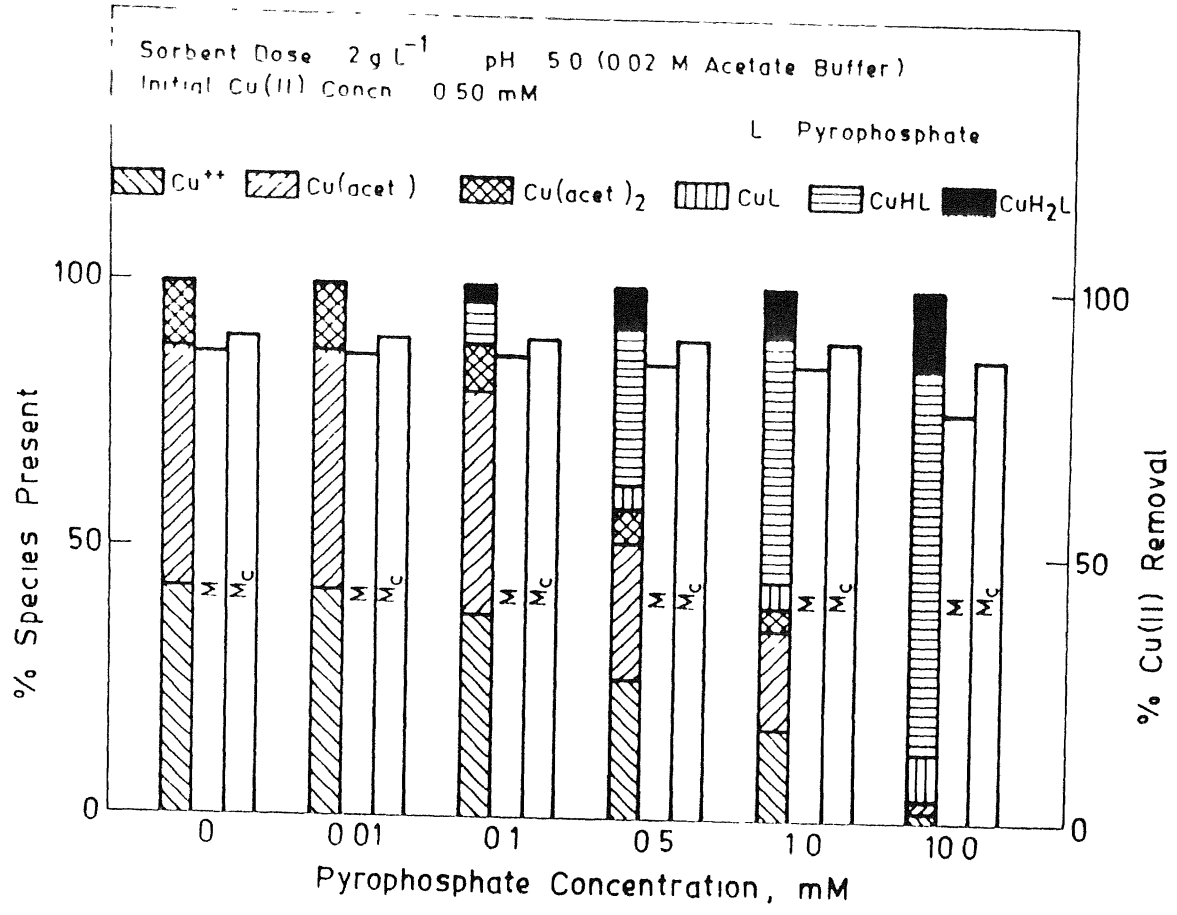

Fig 5 11 Copper Speciation and Their Removal from Aqueous Phase, for Varying Ligand Concn (Pyrophosphate) at pH 5 0

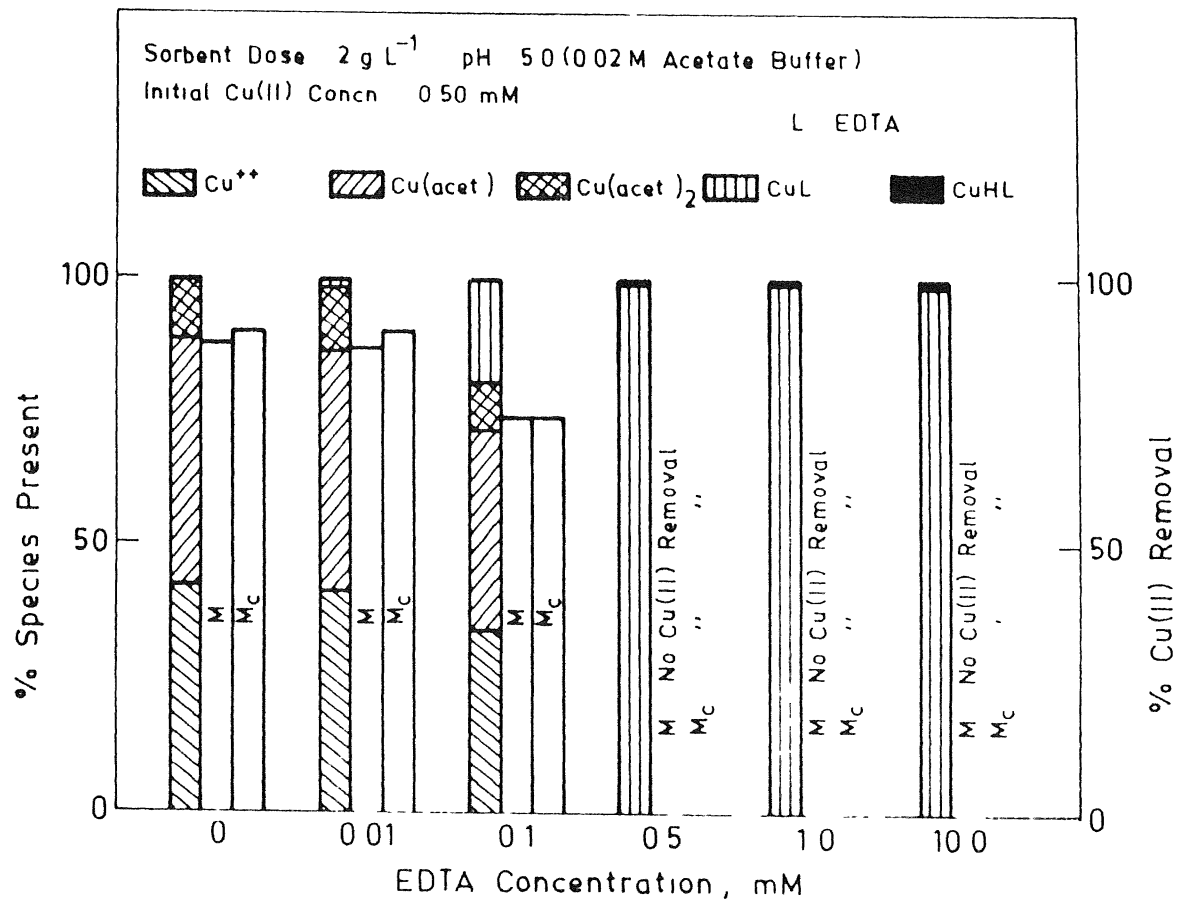

Fig 5 14 Copper Speciation and Their Removal from Aqueous Phase, for Varying Ligand (EDTA) Concn at pH 5 0

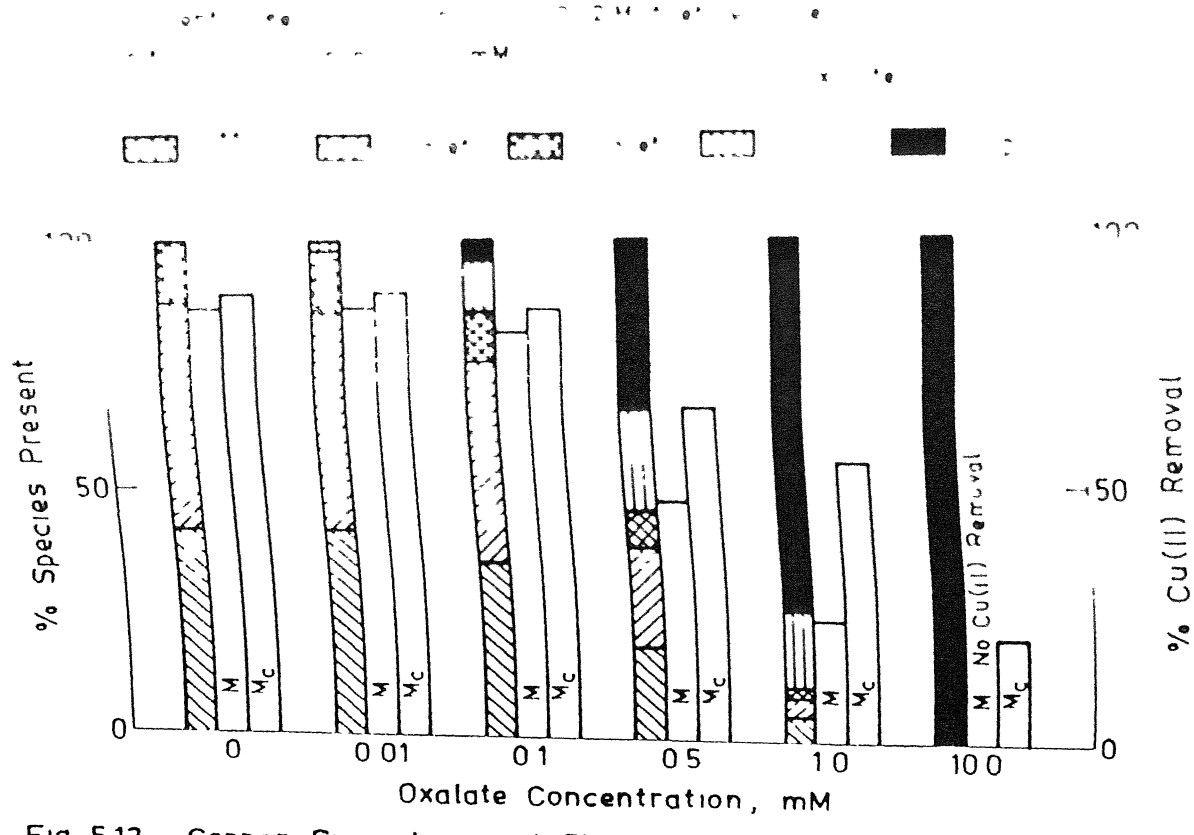

Fig 5 12 Copper Speciation and Their Removal from Aqueous Phase, for Varying Ligand Concn (Oxalate) at pH 5 0

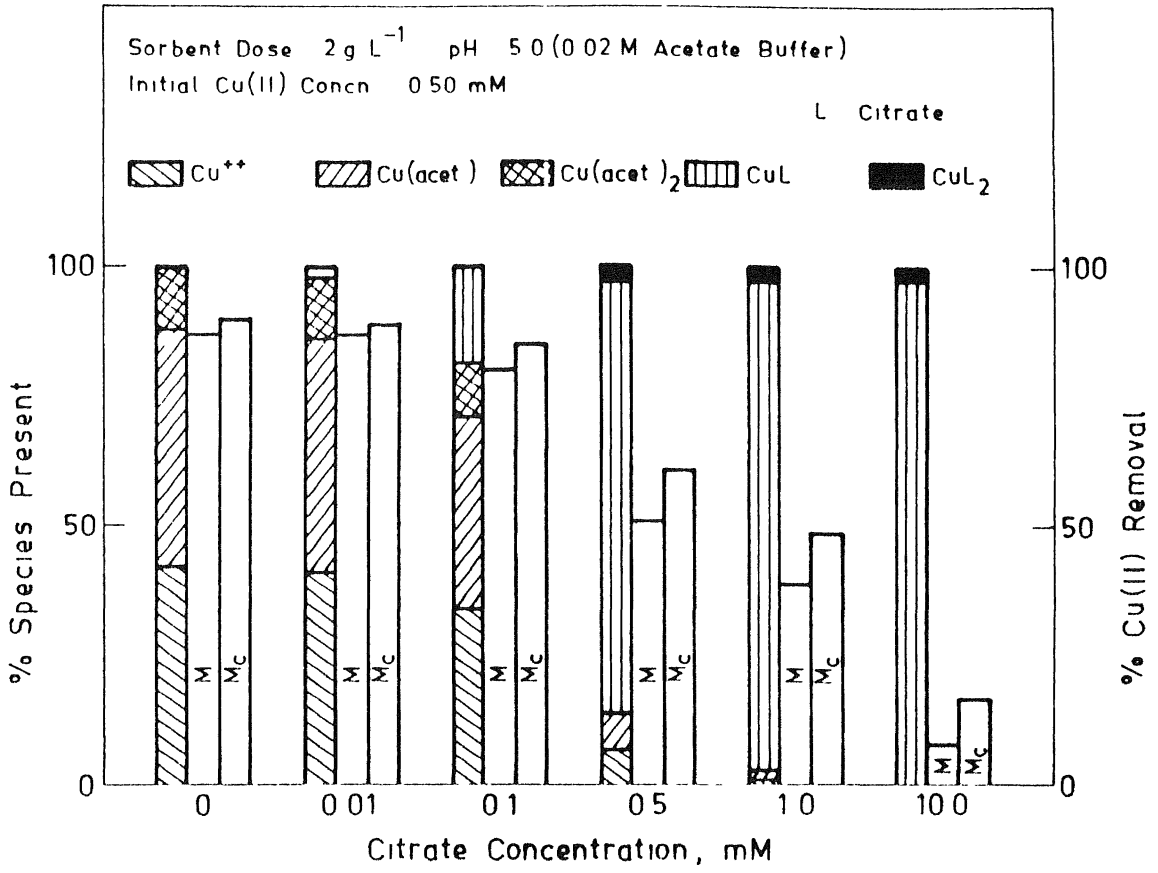

Fig 5 13 Copper Speciation and Their Removal from Aqueous Phase, for Varying Ligand Concn (Citrate) at pH 5 0

of 1:20) exhibited apparently no inhibition in the copper(II) uptake by both M and M_c (Figure 5.11) while EDTA completely inhibited the Cu(II) uptake even at a concentrations of 0.5 mM (metal:ligand of 1.1) as per Figure 5.14.

The metal uptake, in the absence of the ligands but in presence of a common anion (acetate) used to maintain the pH, was taken as a control to compare the inhibition of metal uptake by other ligands. Results show that the ligands upto a concentration of 0.01 mM do not have any effect on the speciation of metal as well as on the metal uptake potential of the sorbents. The dominant species, in above systems as well as the ligand-free system, were free Cu^{++} ions (42%) and copper-acetate (45%). The free metal was cross checked with that measured using orion ion specific electrode. Though Cu-$(acetate)_2$ complex was present (13%) in these systems, it was not removed by M while a small fraction (3%) of this species could be removed by M_c. Further it can be observed that there is a sharp decrease in free metal concentration at ligand concentrations of 0.5 mM, for all the ligands. The free metal was zero for an EDTA concentration of 0.5 mM.

A close examination of the figures shows that the decrease in concentrations of free metal as well as acetate complexes reduced the metal uptake capacity of the biosorbents except in the presence of pyrophosphate. The metal uptake by the biosorbents were completely inhibited by the presence of EDTA at concentrations of 0.5 mM and higher, indicating that the groups on sorbents are not able to free the metal from the

Cu-EDTA complex. The lesser inhibition by pyrophosphate on metal uptake can be attributed to the capacity of the binding sites on the sorbent surface to interact with the copper-pyrophosphate complexes. Both M and M_c removed completely the Cu-pyrophosphate complexes, though it was the dominant species (73%) at a ligand concentration of 10 mM (M:L of 1:20). These results obtained with the pyrophosphate system are significant and indicate that the removal of Cu(II) by biosorption from effluents of metal processing industries could be successfully employed, provided pyrophospnate is used in the metal processing, to keep the metal in solution.

The metal uptake in the presence of 1 mM citrate was found to be 49% and 59% respectively by M and M_c, while it was reduced considerably when the ligand concentration was increased to 10 mM (Figure 5.13).

Treated G. lucidum (M_c) was marginally better than the untreated one in biosorptive capacity in the presence of ligands, except for oxalate, for which it showed a large removal compared to M. When the oxalate concentration was 10 mM, 20% metal uptake was observed with M_c while apparently no removal was observed with M.

The metal uptake inhibition caused by the anions, in general, followed the order, EDTA > oxalate > citrate > pyrophosphate for G. lucidum (M) while it was slightly different for treated G. lucidum, i.e., EDTA > citrate > oxalate > pyrophosphate. It is logical to compare the stability constants of the metal-ligand complex with the uptake inhibition observed in

presence of anions followed the same order of magnitude as that of the stability constants. The deviation from this order, observed for treated G. lucidum suggests the changes in certain surface binding sites which could displace more metal from the copper-oxalate complex. Further, the metal uptake observed with treated G. lucidum, in the presence of oxalate of 10 mM (Figure 5.12), suggests that the binding sites of the sorbents could accumulate Cu(II) even when the dominant species was $Cu(oxalate)_2$ while this was the case by untreated G. lucidum. It appears that in the presence of oxalate and citrate, employing M_c for metal removal is better.

Since no enhancement of the metal uptake was observed either by M or by M_c, in the presence of complexing anions, the metal-sorbent-ligand interactions may be grouped into the following categories i.e., (1) metal ligand complexes may form that are non-adsorbing or weakly adsorbing resulting in a decrease in metal sorption due to the ligand presence and (2) the anionic ligands on the sorbent surface may shift the equilibrium from metal-aqueous ligand complexes to that of metal-sorbent depending on their stability constants.

To know whether these sorbents are able to adsorb the metal-ligand complexes, COD of the aqueous phase was determined before and after the sorption process. No significant change in COD during the sorption process was observed, thus negating the first premise that the metal-ligand complex as such is getting adsorbed. Further, the results of COD are indicative of the shifting of the metal equilibrium from ligand based

aqueous phase to solid based ligand system in which the ligand contributing COD is left behind the liquid.

5.6. Measurement of Complexation Parameters

The complexation reaction between a metal, M, and a ligand, L, can be represented by $nM + L = M_nL$ where n is a positive integer. The equilibrium constant, K, for the reaction is given by

$$K = \frac{[M_nL]}{[M]^n [L]} \qquad (5.7)$$

The complexation capacity and conditional stability constants of the sorbent can be evaluated by adding a range of metal concentrations to it in the absence of other possible competitive inorganic or organic ligands. Determination of conditional stability constants is usually dependant on measurement of the free and complexed (or sorbent bound) metal concentrations, M_f and M_b respectively, with no direct measurement of either the bound or free ligand concentrations (L_b and L_f, respectively). It is to be noted here that the sorbent is considered as a ligand with which the metal is complexed. If the complex has 1:1 stoichiometry, then L_f can be expressed as $(L_t - M_b)$ and M_nL as M_b. Substituting these values into Equation 5.7 and reaa rearranging yields the linear expression from which the conditional stability constants, K' and the complexation capacity, L_t, can be calculated (Ruzic, 1982), i.e.,

$$\frac{M_f}{M_b} = \frac{L_f}{L_t} + \frac{1}{K'L_t} \qquad (5.8)$$

A plot of M_b against M_f yields a rectangular hyperbola

which is often described as "L-shaped". Thus, metal adsorption that correspond to an L-shaped isotherm could be representative of 1:1 complex formation. Further, by plotting the ratio of free to bound metal (M_f/M_b) versus the free metal concentration (M_f), a straight line is obtained for which the slope is the inverse value of the complexation capacity ($1/L_t$) and the intercept is the inverse value of the product of the conditional stability constant and the complexation capacity ($1/K'L_t$).

In the present investigation the biosorbents (M and M_c) were equilibrated with a range of free metal ion concentrations to yield a series of values for M_f and M_b, which were subsequently used for the determination of complexation parameters. Sorbents preconditioned at pH 5 were used so that no other anion need to be incorporated in the sorption process to maintain the constant pH. The free metal ion was determined using orion Cu(II) selective electrode.

Plots of bound metal concentration (M_b) against free metal concentration (M_f) for M and M_c, presented in Figure 5.15, gave typical L-shaped curves indicating 1:1 stoichiometry between the metal and ligand (here sorbent). Further, the L-shaped curve is indicative of a reduction in the number of available binding sites as $[M_f]$ increases, such that the proportion of total metal bound at higher concentrations decreases. Such a phenomena may be described by the Langmuir isotherm.

$$\frac{C_e}{q_e} = \frac{1}{bx_o} + \frac{C_e}{x_o} \qquad (5.9)$$

which is analogous to the equation 5.8. Here the affinity and

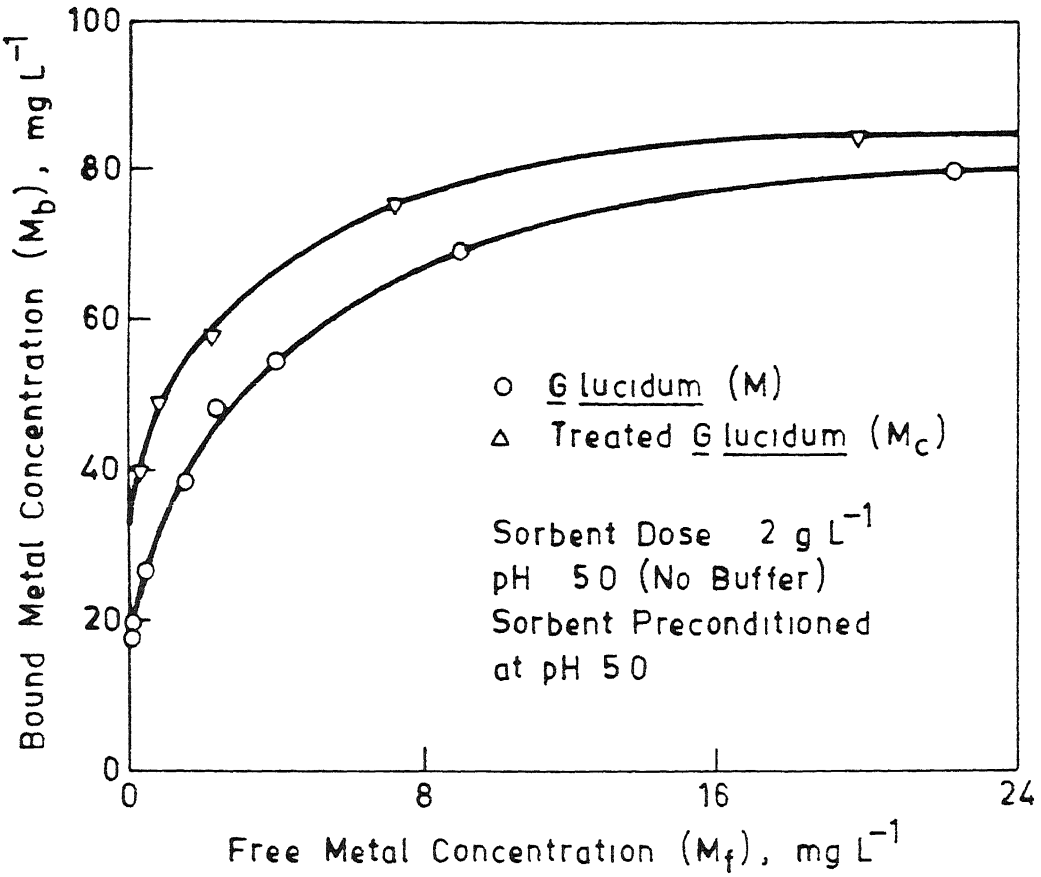

Fig 5 15 Plot of Free Metal Concentration (M_f) to Bound Metal Concentration (M_b)

capacity parameter (b and Q_o, respectively) are equivalent to the conditional stability constants (K') and complexation capacity (L_t) respectively. When this isotherm is applied to adsorption, the following conditions must be met (Lawson et al., 1984): (i) a monolayer of ions is formed on the sorbents, and these ions are non-interactive; (ii) the energy of adsorption should be the same for all ions. When applied to complexation, these conditions may be translated as (i) the formation of complexes with 1:1 stoichiometry and (ii) the identical nature of all binding sites contributing a particular value of $[L_t]$.

The results obtained in the present course of experiment were used for the determination of complexation parameters (K' and L_t) by plotting the ratio (M_f/M_b) Vs. M_f as per Figure 5.16. Untreated G. lucidum appears to have the group(s) with log K' values of 4.5 L/mole while alkali treated G. lucidum has group(s) with log K' 4.7 L/mole. The complexation capacity (L_t) for M and M_c are respectively 670 and 708 umol/g. Both log K' and L_t values are more for M_c than M, thus the former should have higher copper removal potential. Experimentally M_c always registered higher metal removal. Similar approach was employed by Stephenson et al. (1987) to delineate the mechanism of metal removal in activated sludge.

Triamino triethylamine [$(NH_2CH_2CH_2)_3N$, Tren], an amine group has a log K' value of 4.1 with copper at pH 5.0 (Ringbom, 1963). It may be assumed that this amine group present on sorbent may be responsible for copper sorption. However, further proof for this is needed.

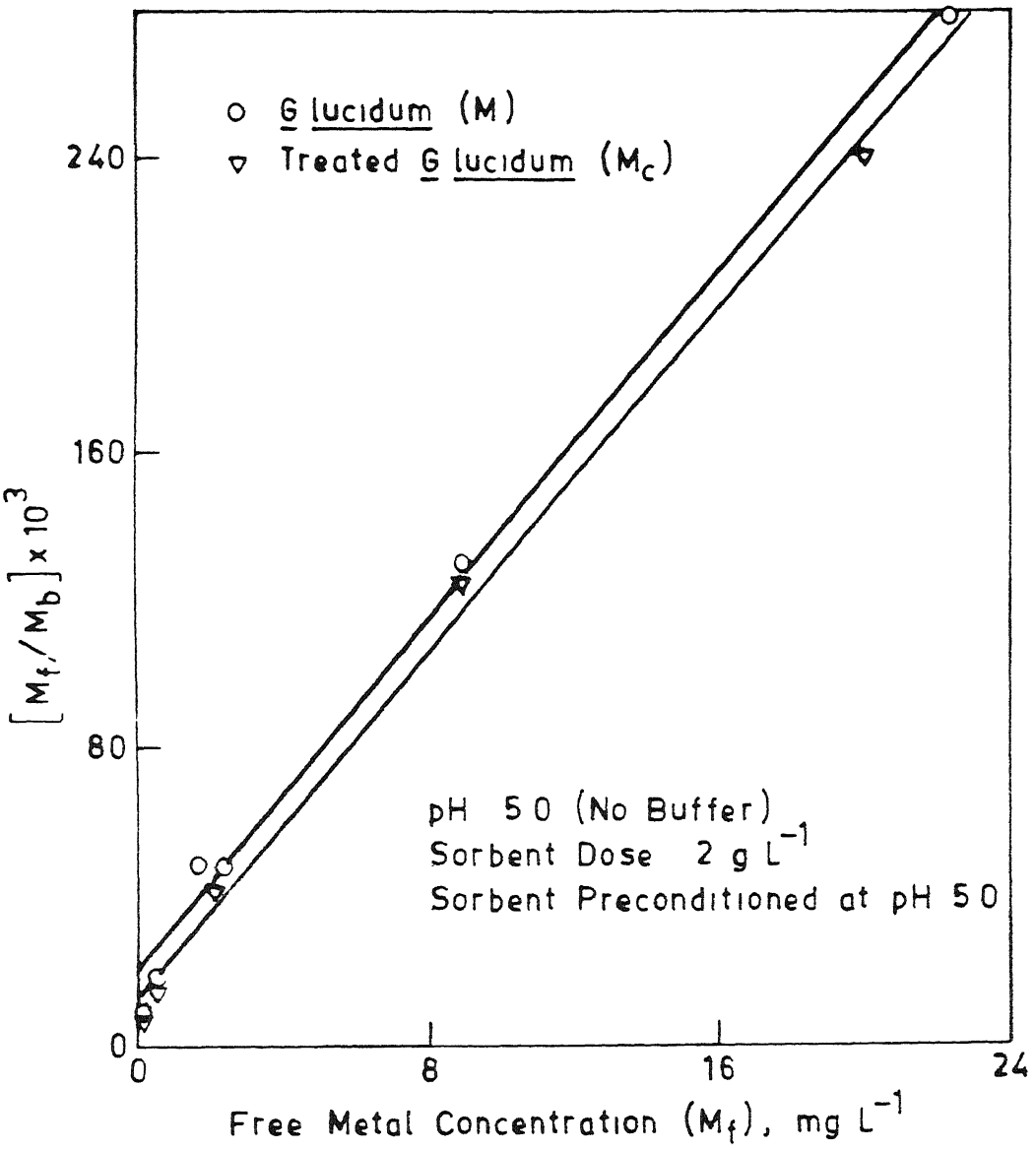

Fig 5.16 Plot of Free Metal Concentration (M_f) vs M_f/M_b.

In the preceding section it was mentioned that pyrophosphate forms a weak complex with copper (log K', 1.1) in aqueous phase at a solution pH of 5.0. Since the groups on M and M_c have a higher log K' values, the metal from pyrophosphate complex is released to get adsorbed to the sorbent group. The uninhibited uptake of metal in the presence of pyrophosphate gives experimental support for this. EDTA forms the stronger complex with copper (log K', 10.7). But, the Cu-sorbent ligand complex is much weaker (log K', 4.5) and hence no metal removal from aqueous phase can be expected in the presence of EDTA. Further, desorption using EDTA yielded almost 100% removal of metal from sorbent indicating EDTA can break the Cu-sorbent ligand complex releasing the Cu(II) into aqueous phase.

The above mentioned model is based on the assumption of 1:1 stoichiometry, while 'n' need not be unity. Stephenson et al. (1987) suggested a model to evaluate log K' values when $n \neq 1$, for metal-ligand interaction. For a metal-ligand system a fractional saturation coefficient, F, can be defined as the metal bound at a stoichiometry of n:1, i.e., $n[M_nL]$, as a proportion of the maximum metal binding capacity, nL_t, i.e.,

$$F = \frac{n[M_nL]}{nL_t} = \frac{[M_nL]}{L_f + [M_nL]} = \frac{K'M_f^n}{1 + K' M_f^n} \qquad (5.10)$$

Setting $K' = 1/K$ yields

$$F = \frac{M_f^n}{K + M_f^n} \qquad (5.11)$$

A linear form of Equation 5.11 is as follows:

$$\frac{F}{1 - F} = \left(\frac{M_f^n}{k + M_f^n}\right)\left(\frac{K + M_f^n}{k}\right) = \frac{M_f^n}{k} \qquad (5.12)$$

Hence

$$\log\left(\frac{F}{1 - F}\right) = n \log M_f - \log K = n \log M_f + \log K' \qquad (5.13)$$

A break-point can be detected in the plot of M_f against M_t, since there will be sharp change in slope of the plot, when the binding sites are fully occupied with the metal. Beyond this break-point, the metal added to the system will remain in uncombined form. If the coordinates of the break-point are denoted (p, q), a parameter 'α' can be calculated from

$$\alpha = p - q \qquad (5.14)$$

For all values of M_t that are less than p, F is given by

$$F = \frac{M_t - M_f}{\alpha} \qquad (5.15)$$

whence

$$\log\left(\frac{M_b}{\alpha - M_b}\right) = n \log M_f + \log K' \qquad (5.16)$$

Equation 5.16 can be solved graphically to yield the stoichiometry of the complex (n) and K' can be calculated from the intercept on y-axis.

Figure 5.17 shows the variation of free metal concentration obtained with M and M_c, over a range of total metal concentration added to the system. The value of 'α' obtained from this plot was used to determine the stoichiometry (n) and conditional ability constants (K') by plotting the values of

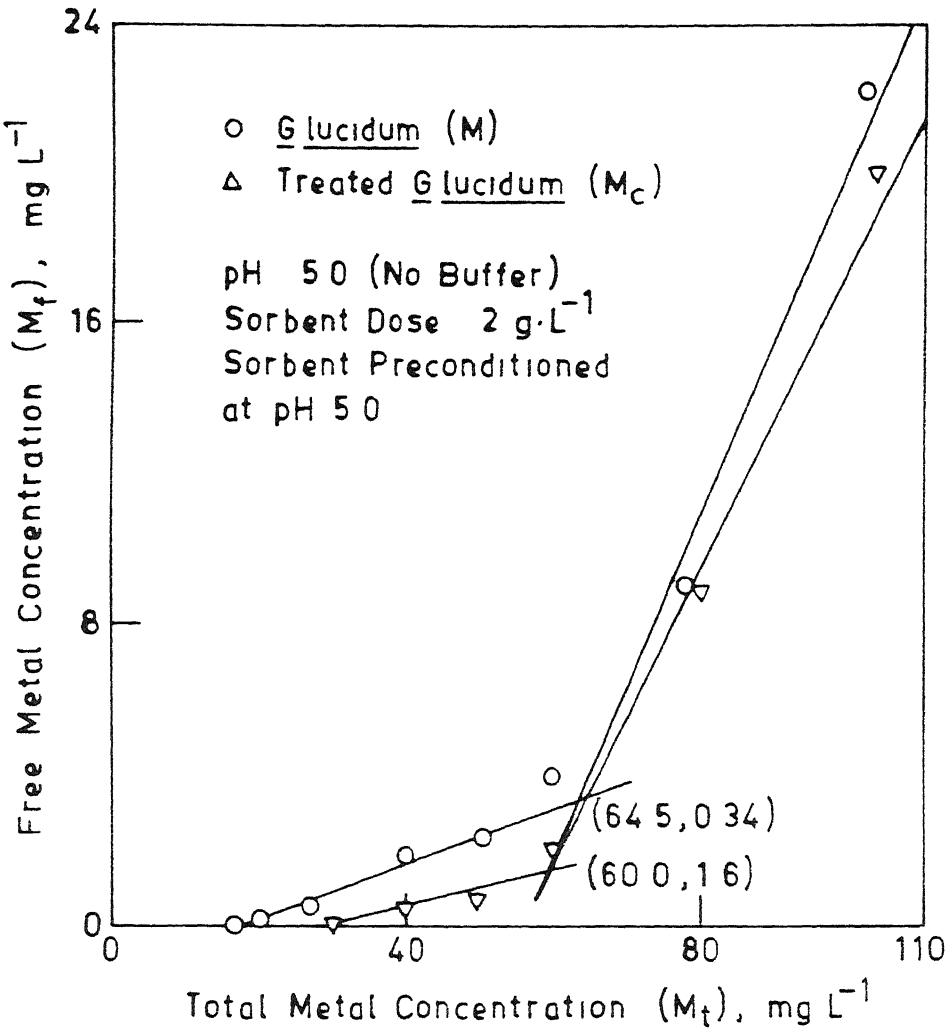

Fig 5 17 Plot of Total Metal Concentration (M_t) vs Free Metal Concentration (M_f)

$\log(\frac{M_b}{a - M_b})$ Vs. the $\log(M_f)$ values as shown in Figure 5.18. The values of 'n' for M and M_c are 0.733 and 0.740 respectively. The conditional stability constants, obtained for M and M_c, assuming an n:1 stoichiometry, are 5.02 and 5.64 L/mol respectively, which are slightly higher than those values for 1:1 stoichiometry. This revised values for log K' indicate slightly higher level of complexation that actually occurs.

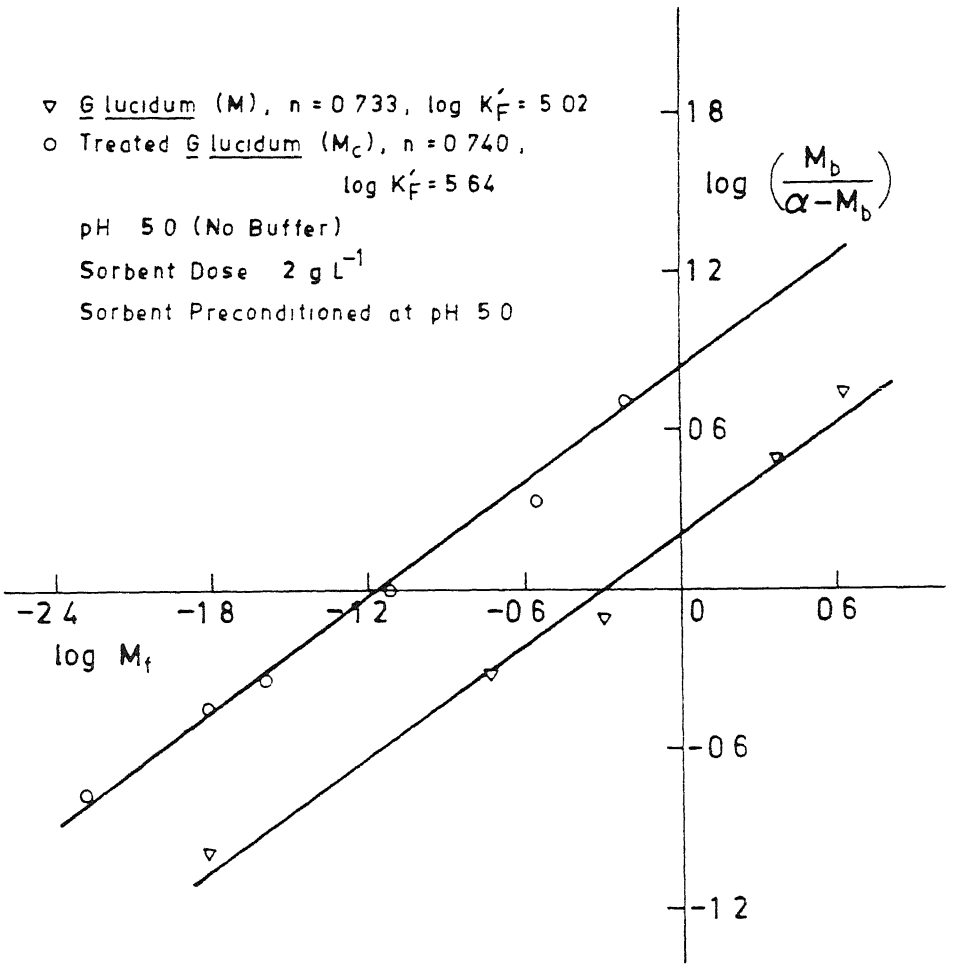

Fig 5 18 Plot of $\log \left(\frac{M_b}{\alpha - M_b}\right)$ vs M_f

6. SUMMARY AND CONCLUSIONS

The effect of various aqueous conditions like pH and of the presence of different complexing ligands usually associated with metals in waste streams, on the biosorption of copper by G. lucidum was investigated. The adsorption-desorption behaviour of the biosorption system was also investigated to determine the reversible component of sorption. The efficiencies of different eluants to desorb the metal from the sorbent were studied. Further an attempt was made to evaluate the complexation capacity and conditional stability constants of the metal binding site(s) or ligand(s) on the surface. Based on the results in the present investigation, the following conclusions may be drawn:

1. Kinetics of biosorption of Cu(II) is very rapid completing 90% of the sorption in 30 minutes and attaining the equilibrium time less than an hour.
2. pH 6 appears to be the optimum for biosorption of Cu(II) by M and M_C. The difference in metal uptake is marginal at pH 5 and 6. Usually the pH of metal processing wastewaters ranges from 5 to 6 and hence, G. lucidum can be effectively employed for metal removal from effluents of metal processing industries. The anions presented in the buffer affect the sorption process. Out of acetate and phthalate buffer, the later exhibited less removal. The removal by sorbents, preconditioned at pH was same as when acetate buffer was used.

3. The biosorption of Cu(II) by M and M_c is a non-reversible process. Hence the metal removal may be due to the chemical bonding between the metal and the ligand(s) on surface of the sorbents.

4. The presence of anions in the solution was found to inhibit the uptake of Cu(II) by M and M_c. The extent of uptake inhibition is related to the metal ligand binding strength. In pyrophospnate system, the inhibition to metal uptake is the least while for EDTA, it is the maximum. Hence the use of pyrophospnate as a complexing agent to keep the metal in solution in metal plating industries, can definitely help the end pipe pollution control methods, as the metal can be removed/recovered efficiency. M_c exhibited better metal uptake than M in the presence of oxalate.

5. Solutions of HCl and EDTA, appears to be efficient eluants, capable of desorbing almost 100% of the sequestered copper. The elution efficiency of EDTA can be attributed to its strong chelating capability (stability constants =10.7).Furthe complete inhibition of metal uptake in the presence of EDTA by M and M_c provide a strong evidence to its chelating efficiency.

6. The conditional stability constants and complexation capacities obtained for the site(s) or ligand(s) on the sorbent surface, suggest the existence of certain group on the surface which have similar complexation capacity as that of an amine complex (Tren). This conditional stability constants (4.5 and 4.7 L/mole respectively for M

and M_c) are indicative of their potential to adsorb the metal from aqueous phase when pyrophosphate, oxalate or citrate ions are present along with the metal in solution and failure to do so when EDTA is present.

7. SUGGESTIONS FOR FUTURE WORK

The following suggestions may be made for future work which include

1. Precise identification of the anionic ligand(s)/group(s) on the sorbent surface so that the mechanism of biosorption of Cu(II) can be elucidated.
2. Studies on the effect of organic or inorganic ligands on the sorption process by employing a continuous flow system.
3. Studies on multi-metal and multi-ligand system to evaluate the potential of G. lucidum for removal of these metals.
4. Optimisation of elution process using different concentration and volumes of eluants.
5. Batch and continuous studies on metal removal from actual wastewater from industries.

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